



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ :		A2	(11) International Publication Number:	WO 95/23225
C12N 15/52, 9/00, A61K 31/70, C07H 19/04, 19/10, 19/20, C12N 15/10, A61K 48/00, C12N 15/86, 15/87			(43) International Publication Date:	31 August 1995 (31.08.95)
(21) International Application Number:		PCT/IB95/00156		
(22) International Filing Date:		23 February 1995 (23.02.95)		
(30) Priority Data:				
08/201,109	23 February 1994 (23.02.94)	US	(72) Inventors:	
08/218,934	29 March 1994 (29.03.94)	US	STINCHCOMB, Dan, T.; 7203 Old Post Road, Boulder, CO 80301 (US). CHOWRIRA, Bharat;	
08/222,795	4 April 1994 (04.04.94)	US	3250 O'Neal Circle, B-25, Boulder, CO 80301 (US). DIRENZO, Anthony; 1197 Ravenwood Road, Boulder, CO 80303 (US).	
08/224,483	7 April 1994 (07.04.94)	US	DRAPER, Kenneth, G.; 4619 Cloud Ct., Boulder, CO 80301 (US). DUDYJCZ, Lech, W.; 24 A Gates Road, Worcester, MA 01603 (US). GRIMM, Susan; 6968 1/2 S. Boulder	
08/228,041	15 April 1994 (15.04.94)	US	Road, Boulder, CO 80303 (US). KARPEISKY, Alexander; 5121 Williams Fork Trail #209, Boulder, CO 80301 (US).	
08/227,958	15 April 1994 (15.04.94)	US	KISICH, Kevin; 2451 Jonquil Circle, Lafayette, CO 80026 (US). MATULIC-ADAMIC, Jasenka; 760 South 42nd	
08/245,736	18 May 1994 (18.05.94)	US	Street, Boulder, CO 80303 (US). McSWIGGEN, James, A.; 4866 Franklin Drive, Boulder, CO 80301 (US). MODAK,	
08/271,280	6 July 1994 (06.07.94)	US	Anil; 3855 Hauptman Court, Boulder, CO 80301 (US).	
08/291,932	15 August 1994 (15.08.94)	US	PAVCO, Pamela; 705 Barberry Circle, Lafayette, CO 80026 (US). BEIGELMAN, Leonid; 5530 Colt Drive, Longmont, CO 80503 (US). SULLIVAN, Sean, M.; 850 Marina	
08/291,433	16 August 1994 (16.08.94)	US	Village Parkway, Alameda, CA 94501 (US). SWEEDLER, David; 956 St. Andrews Lane, Louisville, CO 80027	
08/292,620	17 August 1994 (17.08.94)	US	(US). THOMPSON, James, D.; 2925 Glenwood Drive #301, Boulder, CO 80301 (US). TRACZ, Danuta; 6200	
08/293,520	19 August 1994 (19.08.94)	US	Habitat #3029, Boulder, CO 80301 (US). USMAN, Nassim; 2954 Kalmia #37, Boulder, CO 80304 (US). WINCOTT, Francine, E.; 7920 N. 95th Street, Longmont, CO 80501 (US). WOOLF, Tod; 18 Fairview Avenue, Watertown, MA 02172 (US).	
08/300,000	2 September 1994 (02.09.94)	US	(74) Agents:	
08/303,039	8 September 1994 (08.09.94)	US	WARBURG, Richard, J. et al.; Lyon & Lyon, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).	
08/311,486	23 September 1994 (23.09.94)	US	(81) Designated States:	
08/311,749	23 September 1994 (23.09.94)	US	AU, CA, JP, KR, MX, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
08/314,397	28 September 1994 (28.09.94)	US	Published	
08/316,771	3 October 1994 (03.10.94)	US	<i>Without international search report and to be republished upon receipt of that report.</i>	
08/319,492	7 October 1994 (07.10.94)	US		
08/321,993	11 October 1994 (11.10.94)	US		
08/334,847	4 November 1994 (04.11.94)	US		
08/337,608	10 November 1994 (10.11.94)	US		
08/345,516	28 November 1994 (28.11.94)	US		
08/357,577	16 December 1994 (16.12.94)	US		
08/363,233	23 December 1994 (23.12.94)	US		
08/380,734	30 January 1995 (30.01.95)	US		
(71) Applicant: RIBOZYME PHARMACEUTICALS, INC. [US/US]; 2950 Wilderness Place, Boulder, CO 80301 (US).				
(54) Title: METHOD AND REAGENT FOR INHIBITING THE EXPRESSION OF DISEASE RELATED GENES				

(57) Abstract

Enzymatic RNA molecules which cleave ICAM-I mRNA, IL-5 mRNA, *rel A* mRNA, TNF- α mRNA, RSV mRNA or RSV genomic RNA, or CML associated mRNA, and use of these molecules for the treatment of pathological conditions related to those mRNA-levels; ribonucleosides or nucleotides modified in 2', 3' or 5', methods for their synthesis, purification and deprotection; vectors containing multiple enzymatic nucleic acids, optionally in chimeric form with tRNAs; method for introducing enzymatic nucleic acids into cells by forming a complex with a second nucleic acid, where the complex is capable of taking an R-loop base-paired structure; method for altering a mutant nucleic acid *in vivo* by hybridization with an oligonucleotide capable of activating dsRNA deaminase, comprising an enzymatic activity or a chemical mutagen. Further are disclosed trans-cleaving or -ligating hairpin ribozymes lacking a substrate RNA moiety, as well as hammerhead ribozymes having an interconnecting loop between base pairs in stem II.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

METHOD AND REAGENT FOR INHIBITING THE EXPRESSION
OF DISEASE RELATED GENESBackground of the Invention

This invention relates to reagents useful as inhibitors of gene expression relating to diseases such as inflammatory or autoimmune disorders, chronic myelogenous leukemia, or respiratory tract illness.

5

Summary of the Invention

The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting the expression of disease related genes, e.g., ICAM-1, IL-5, relA, TNF- α , p210 bcr-abl, and respiratory syncytial virus genes. Such ribozymes can be used in a method for 10 treatment of diseases caused by the expression of these genes in man and other animals, including other primates.

Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. Such enzymatic RNA molecules can be 15 targeted to virtually any RNA transcript, and efficient cleavage has been achieved *in vitro*. Kim et al., 84 Proc. Natl. Acad. Sci. USA 8788, 1987; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acids Research 1371, 1989.

Six basic varieties of naturally-occurring enzymatic RNAs are known 20 presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. Table 1 summarizes some of the characteristics of these ribozymes.

Ribozymes act by first binding to a target RNA. Such binding occurs 25 through the target RNA binding portion of a ribozyme which is held in close proximity to an enzymatic portion of the RNA which acts to cleave the target RNA. Thus, the ribozyme first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a 30 target RNA will destroy its ability to direct synthesis of an encoded protein. After a ribozyme has bound and cleaved its RNA target it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

- The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the effective concentration of ribozyme necessary to effect a therapeutic treatment is lower than that of an antisense oligonucleotide. The advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding, but also on the mechanism by which the molecule inhibits the expression of the RNA to which it binds. That is, the inhibition is caused by cleavage of the RNA target and so specificity is defined as the ratio of the rate of cleavage of the targeted RNA over the rate of cleavage of non-targeted RNA. This cleavage mechanism is dependent upon factors additional to those involved in base pairing. Thus, it is thought that the specificity of action of a ribozyme is greater than that of antisense oligonucleotide binding the same RNA site. With their catalytic activity and increased site specificity, ribozymes represent more potent and safe therapeutic molecules than antisense oligonucleotides.
- Thus, in a first aspect, this invention relates to ribozymes, or enzymatic RNA molecules, directed to cleave RNA species encoding ICAM-1, IL-5, relA, TNF- α , p210bcr-abl, or RSV proteins. In particular, applicant describes the selection and function of ribozymes capable of cleaving these RNAs and their use to reduce levels of ICAM-1, IL-5, relA, TNF- α , p210 bcr-abl or RSV proteins in various tissues to treat the diseases discussed herein. Such ribozymes are also useful for diagnostic uses.
- Applicant indicates that these ribozymes are able to inhibit expression of ICAM-1, IL-5, rel A, TNF- α , p210bcr-abl, or RSV genes and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave target ICAM-1, IL-5, rel A, TNF- α , p210bcr-abl, or RSV encoding mRNAs may be readily designed and are within the invention.
- These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the

cleavage of RNA. Upon binding, the ribozymes cleave the target encoding mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

By "gene" is meant to refer to either the protein coding regions of the 5 cognate mRNA, or any regulatory regions in the RNA which regulate synthesis of the protein or stability of the mRNA; the term also refers to those regions of an mRNA which encode the ORF of a cognate polypeptide product, and the proviral genome.

By "enzymatic RNA molecule" it is meant an RNA molecule which has 10 complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave RNA in that target. That is, the enzymatic RNA molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the 15 enzymatic RNA molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. By "equivalent" RNA to a virus is meant to include those naturally occurring viral encoded RNA molecules associated with viral caused diseases in various animals, 20 including humans, cats, simians, and other primates. These viral or viral-encoded RNAs have similar structures and equivalent genes to each other.

By "complementarity" it is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example, Hoogsteen type) of base-paired interactions. 25

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. 30 Examples of such hammerhead motifs are described by Rossi *et al.*, 1992, *Aids Research and Human Retroviruses*, 8,183, of hairpin motifs by Hampel and Tritz, 1989 *Biochemistry*, 28, 4929, EP 0360257 and Hampel et al., 1990, *Nucleic Acids Res.* 18,299 and an example of the hepatitis delta virus motif is described by Perotta and Been, 1992 *Biochemistry*, 31 35 16 of the RNaseP motif by Guerrier-Takada *et al.*, 1983 *Cell*, 35 849,

cleavage of RNA. Upon binding, the ribozymes cleave the target encoding mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

By "gene" is meant to refer to either the protein coding regions of the 5 cognate mRNA, or any regulatory regions in the RNA which regulate synthesis of the protein or stability of the mRNA; the term also refers to those regions of an mRNA which encode the ORF of a cognate polypeptide product, and the proviral genome.

By "enzymatic RNA molecule" it is meant an RNA molecule which has 10 complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave RNA in that target. That is, the enzymatic RNA molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the 15 enzymatic RNA molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. By "equivalent" RNA to a virus is meant to include those naturally occurring viral encoded RNA molecules associated with viral caused diseases in various animals, 20 including humans, cats, simians, and other primates. These viral or viral-encoded RNAs have similar structures and equivalent genes to each other.

By "complementarity" it is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example, Hoogsteen type) of base-paired interactions.

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. 30 Examples of such hammerhead motifs are described by Rossi *et al.*, 1992, *Aids Research and Human Retroviruses*, 8,183, of hairpin motifs by Hampel and Tritz, 1989 *Biochemistry*, 28, 4929, EP 0360257 and Hampel et al., 1990, *Nucleic Acids Res.* 18,299 and an example of the hepatitis delta virus motif is described by Perotta and Been, 1992 *Biochemistry*, 31 16 of the RNaseP motif by Guerrier-Takada et al., 1983 *Cell*, 35 849,

expressed in eukaryotic cells from the appropriate DNA or RNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO93/23569, and Sullivan et al., PCT WO94/02595, both hereby incorporated in their totality by reference herein; Ohkawa, J., et al., 1992, Nucleic Acids Symp. Ser. 27, 15-6; Taira, K. et al., Nucleic Acids Res., 19, 5125-30; Ventura, M., et al., 1993, Nucleic Acids Res., 21, 3249-55, Chowrira et al., 1994 J. Biol. Chem. 269, 25856).

By "inhibit" is meant that the activity or level of ICAM-1, Rel A, IL-5, TNF- α , p210bcr-abl or RSV encoding mRNA is reduced below that observed in the absence of the ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the level of ICAM-1, IL-5, Rel A, TNF- α , p210bcr-abl or RSV protein or activity in a cell or tissue. By "related" is meant that the inhibition of ICAM-1, IL-5, Rel A, TNF- α , p210bcr-abl or RSV mRNA translation, and thus reduction in the level of, ICAM-1, IL-5, Rel A, TNF- α , p210bcr-abl or RSV proteins will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues through the use of a catheter, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 2,3,6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36 and 37.

Examples of such ribozymes are shown in Tables 4-8, 10, 12, 14-16, 19-22, 24, 26-28, 30, 32, 34 and 36-38. Examples of such ribozymes consist essentially of sequences defined in these Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in 5 the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of 10 two base-paired stem structure can form. The sequence listed in the above identified Tables may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

In another aspect of the invention, ribozymes that cleave target 15 molecules and inhibit ICAM-1, IL-5, Rel A, TNF- α , p210bcr-abl or RSV gene expression are expressed from transcription units inserted into DNA, RNA, or viral vectors. Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA or RNA expression vector. Transcription of the 20 ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, 25 silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 *Proc. Natl. Acad. Sci. USA*, 87, 6743-7; Gao and Huang 1993 *Nucleic Acids Res.*, 21 2867-72; Lieber et al., 1993 *Methods Enzymol.*, 217, 47-66; Zhou et al., 30 1990 *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 *Antisense Res. Dev.*, 2, 3-15; Ojwang et al., 1992 *Proc. Natl. Acad. Sci. USA*, 90, 6340-4; L'Huiller et al., 1992 *EMBO J.* 11, 4411-8; Lisziewicz et al., 1993 *Proc. Natl. 35 Acad. Sci. U.S.A.*, 90 8000-4). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors

(such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from
5 the claims.

Description Of The Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 is a diagrammatic representation of the hammerhead
10 ribozyme domain known in the art. Stem II can be ≥ 2 base-pair long.

Figure 2(a) is a diagrammatic representation of the hammerhead
ribozyme domain known in the art; Figure 2(b) is a diagrammatic
representation of the hammerhead ribozyme as divided by Uhlenbeck
(1987, *Nature*, 327, 596-600) into a substrate and enzyme portion; Figure
15 2(c) is a similar diagram showing the hammerhead divided by Haseloff and
Gerlach (1988, *Nature*, 334, 585-591) into two portions; and Figure 2(d) is
a similar diagram showing the hammerhead divided by Jeffries and
Symons (1989, *Nucl. Acids. Res.*, 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a
20 hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, n
is 1,2,3 or 4) and helix 5 can be optionally provided of length 2 or more
bases (preferably 3-20 bases, *i.e.*, m is from 1-20 or more). Helix 2 and
helix 5 may be covalently linked by one or more bases (*i.e.*, r is ≥ 1 base).
Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4-20
25 base pairs) to stabilize the ribozyme structure, and preferably is a protein
binding site. In each instance, each N and N' independently is any normal
or modified base and each dash represents a potential base-pairing
interaction. These nucleotides may be modified at the sugar, base or
phosphate. Complete base-pairing is not required in the helices, but is
30 preferred. Helix 1 and 4 can be of any size (*i.e.*, o and p is each
independently from 0 to any number, *e.g.* 20) as long as some base-pairing
is maintained. Essential bases are shown as specific bases in the
structure, but those in the art will recognize that one or more may be

modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, i.e., without a connecting loop. The connecting loop when present may be a ribonucleotide with or without
5 modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases.
"—" refers to a covalent bond.

Figure 4 is a representation of the general structure of the hepatitis
10 delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a diagrammatic representation of the genetic map of RSV strain A2.

15 Figure 7 is a diagrammatic representation of the solid-phase synthesis of RNA.

Figure 8 is a diagrammatic representation of exocyclic amino protecting groups for nucleic acid synthesis.

Figure 9 is a diagrammatic representation of the deprotection of RNA.

20 Figure 10 is a graphical representation of the cleavage of an RNA substrate by ribozymes synthesized, deprotected and purified using the improved methods described herein.

25 Figure 11 is a schematic representation of a two pot deprotection protocol. Base deprotection is carried out with aqueous methyl amine at 65 °C for 10 min. The sample is dried in a speed-vac for 2-24 hours depending on the scale of RNA synthesis. Silyl protecting group at the 2'-hydroxyl position is removed by treating the sample with 1.4 M anhydrous HF at 65°C for 1.5 hours.

30 Figure 12 is a schematic representation of a one pot deprotection of RNA synthesized using RNA phosphoramidite chemistry. Anhydrous methyl amine is used to deprotect bases at 65°C for 15 min. The sample is allowed to cool for 10 min before adding TEA•3HF reagent, to the same

pot, to remove protecting groups at the 2'-hydroxyl position. The deprotection is carried out for 1.5 hours.

Figs. 13a - b is a HPLC profile of a 36 nt long ribozyme, targeted to site B. The RNA is deprotected using either the two pot or the one pot 5 deprotection protocol. The peaks corresponding to full-length RNA is indicated. The sequence for site B is CCUGGGCCAGGGAUUA AUGGAGAUGCCCCACU.

Figure 14 is a graph comparing RNA cleavage activity of ribozymes deprotected by two pot vs one pot deprotection protocols.

10 Figure 15 is a schematic representation of an improved method of synthesizing RNA containing phosphorothioate linkages.

Figure 16 shows RNA cleavage reaction catalyzed by ribozymes containing phosphorothioate linkages. Hammerhead ribozyme targeted to site C is synthesized such that 4 nts at the 5' end contain phosphorothioate 15 linkages. P=O refers to ribozyme without phosphorothioate linkages. P=S refers to ribozyme with phosphorothioate linkages. The sequence for site C is UCAUUUUGGCCAUCUC UUCCUUCAGGCGUGG.

Figure 17 is a schematic representation of synthesis of 2'-N-phtalimido-nucleoside phosphoramidite.

20 Figure 18 is a diagrammatic representation of a prior art method for the solid-phase synthesis of RNA using silyl ethers, and the method of this invention using SEM as a 2'-protecting group.

Figure 19 is a diagrammatic representation of the synthesis of 2'-SEM-protected nucleosides and phosphoramidites useful for the synthesis 25 of RNA. B is any nucleotide base as exemplified in the Figure, P is purine and I is inosine. Standard abbreviations are used throughout this application, well known to those in the art.

Figure 20 is a diagrammatic representation of a prior art method for deprotection of RNA using TBDMS protection of the 2'-hydroxyl group.

30 Figure 21 is a diagrammatic representation of the deprotection of RNA having SEM protection of the 2'-hydroxyl group.

Figure 22 is a representation of an HPLC chromatogram of a fully deprotected 10-mer of uridylic acid.

Figs. 23 - 25 are diagrammatic representations of hammerhead, hairpin or hepatitis delta virus ribozyme containing self-processing RNA transcript. Solid arrows indicate self-processing sites. Boxes indicate the sites of nucleotide substitution. Solid lines are drawn to show the binding sites of primers used in a primer-extension assay. Lower case letters indicate vector sequence present in the RNA when transcribed from a *Hind*III-linearized plasmid. (23) HH Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hammerhead ribozyme. The structure of the hammerhead ribozyme is based on phylogenetic and mutational analysis (reviewed by Symons, 1992 *supra*). The trans ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (UC at positions 50 and 51) at its 3' end. The 3' processing ribozyme is comprised of nucleotides 44 through 96. Roman numerals I, II and III, indicate the three helices that contribute to the structure of the 3' cis-acting hammerhead ribozyme (Hertel et al., 1992 *Nucleic Acids Res.* 20, 3252). Substitution of G₇₀ and A₇₁ to U and G respectively, inactivates the hammerhead ribozyme (Ruffner et al., 1990 *Biochemistry* 29, 10695) and generates the HH(mutant) construct. (24) HP Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hairpin ribozyme. The structure of the hairpin ribozyme is based on phylogenetic and mutational analysis (Berzal-Herranz et al., 1993 *EMBO J.* 12, 2567). The trans-ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 5 non-ribozyme nucleotides (UGGCA at positions 50 to 54) at its 3' end. The 3' cis-acting ribozyme is comprised of nucleotides 50 through 115. The transcript named HP(GU) was constructed with a potential wobble base pair between G₅₂ and U₇₇; HP(GC) has a Watson-Crick base pair between G₅₂ and C₇₇. A shortened helix 1 (5 base pairs) and a stable tetraloop (GAAA) at the end of helix 1 was used to connect the substrate with the catalytic domain of the hairpin ribozyme (Feldstein & Bruening, 1993 *Nucleic Acids Res.* 21, 1991; Altschuler et al., 1992 *supra*). (25) HDV Cassette, transcript containing the trans-acting hammerhead ribozyme linked to a 3' cis-acting hepatitis delta virus (HDV) ribozyme. The secondary structure of the HDV ribozyme is as proposed by Been and

coworkers (Been et al., 1992 *Biochemistry* 31, 11843). The trans-ribozyme domain extends from nucleotides 1 through 48. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (AA at positions 49 to 50) at its 3' end. The 3' cis-acting HDV ribozyme is comprised of 5 nucleotides 50 through 114. Roman numerals I, II, III & IV, indicate the location of four helices within the 3' cis-acting HDV ribozyme (Perrotta & Been, 1991 *Nature* 350, 434). The ΔHDV transcript contains a 31 nucleotide deletion in the HDV portion of the transcript (nucleotides 84 through 115 deleted).

10 Fig. 26 is a schematic representation of a plasmid containing the insert encoding self-processing cassette. The figure is not drawn to scale.

Fig. 27 demonstrates the effect of 3' flanking sequences on RNA self-processing *in vitro*. H, Plasmid templates linearized with *Hind*III restriction enzyme. Transcripts from H templates contain four non-ribozyme 15 nucleotides at the 3' end. N, Plasmid templates linearized with *Nde*I restriction enzyme. Transcripts from N templates contain 220 non-ribozyme nucleotides at the 3' end. R, Plasmid templates linearized with *Rca*I restriction enzyme. Transcripts from R templates contain 450 non-ribozyme nucleotides at the 3' end.

20 Fig. 28 shows the effect of 3' flanking sequences on the trans-cleavage reaction catalyzed by a hammerhead ribozyme. A 622 nt internally-labeled RNA (<10 nM) was incubated with ribozyme (1000 nM) under single turn-over conditions (Herschlag and Cech, 1990 *Biochemistry* 29, 10159). HH+2, HH+37, and HH+52 are trans-acting ribozymes 25 produced by transcription from the HH, ΔHDV, and HH(mutant) constructs, respectively, and that contain 2, 37 and 52 extra nucleotides on the 3' end. The plot of the fraction of uncleaved substrate versus time was fit to a double exponential curve using the KaleidaGraph graphing program (Synergy Software, Reading, PA). A double exponential curve fit was used 30 because the data points did not fall on a single exponential curve, presumably due to varying conformers of ribozyme and/or substrate RNA.

Fig. 29 shows RNA self-processing in OST7-1 cells. *In vitro* lanes contain full-length, unprocessed transcripts that were added to cellular lysates prior to RNA extraction. These RNAs were either pre-incubated 35 with MgCl₂ (+) or with DEPC-treated water (-) prior to being hybridized

with 5' end-labeled primers. Cellular lanes contain total cellular RNA from cells transfected with one of the four self-processing constructs. Cellular RNA are probed for ribozyme expression using a sequence specific primer-extension assay. Solid arrows indicate the location of primer extension bands corresponding to Full-Length RNA and 3' Cleavage Products.

Figs. 30,31 are diagrammatic representations of self-processing cassettes that will release trans-acting ribozymes with defined, stable stem-loop structures at the 5' and the 3' end following self-processing. 30, shows various permutations of a hammerhead self-processing cassette. 31, 10 shows various permutations of a hairpin self-processing cassette.

Figs. 32a-b Schematic representation of RNA polymerase III promoter structure. Arrow indicates the transcription start site and the direction of coding region. A, B and C, refer to consensus A, B and C box promoter sequences. I, refers to intermediate cis-acting promoter sequence. PSE, 15 refers to proximal sequence element. DSE, refers to distal sequence element. ATF, refers to activating transcription factor binding element. ?, refers to cis-acting sequence element that has not been fully characterized. EBER, Epstein-Barr-virus-encoded-RNA. TATA is a box well known in the art.

20 Figs. 33a-e Sequence of the primary tRNA_imet and Δ3-5 transcripts. The A and B box are internal promoter regions necessary for pol III transcription. Arrows indicate the sites of endogenous tRNA processing. The Δ3-5 transcript is a truncated version of tRNA wherein the sequence 3' of B box has been deleted (Adeniyi-Jones et al., 1984 *supra*). This 25 modification renders the Δ 3-5 RNA resistant to endogenous tRNA processing.

Figure 34. Schematic representation of RNA structural motifs inserted into the Δ3-5 RNA. Δ3-5/HHI- a hammerhead (HHI) ribozyme was cloned at the 3' region of Δ3-5 RNA; S3- a stable stem-loop structure was 30 incorporated at the 3' end of the Δ3-5/HHI chimera; S5- stable stem-loop structures were incorporated at the 5' and the 3' ends of Δ3-5/HHI ribozyme chimera; S35- sequence at the 3' end of the Δ3-5/HHI ribozyme chimera was altered to enable duplex formation between the 5' end and a complementary 3' region of the same RNA; S35Plus- in addition to 35 structural alterations of S35, sequences were altered to facilitate additional

duplex formation within the non-ribozyme sequence of the Δ3-5/HHI chimera.

Figures 35 and 36. Northern analysis to quantitate ribozyme expression in T cell lines transduced with Δ3-5 vectors. 35) Δ3-5/HHI and 5 its variants were cloned individually into the DC retroviral vector (Sullenger et al., 1990 *supra*). Northern analysis of ribozyme chimeras expressed in MT-2 cells was performed. Total RNA was isolated from cells (Chomczynski & Sacchi, 1987 *Analytical Biochemistry* 162, 156-159), and transduced with various constructs described in Fig. 34. Northern analysis 10 was carried out using standard protocols (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). Nomenclature is same as in Figure 34. This assay measures the level of expression from the type 2 pol III promoter. 36) Expression of S35 constructs in MT2 cells. S35 (+ribozyme), S35 construct containing HHI ribozyme. S35 (-ribozyme), S35 construct 15 containing no ribozyme.

Figure 37. Ribozyme activity in total RNA extracted from transduced MT-2 cells. Total RNA was isolated from cells transduced with Δ3-5 constructs described in Figs. 35 and 36. In a standard ribozyme cleavage reaction, 5 µg total RNA and trace amounts of 5' terminus-labeled ribozyme 20 target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris-HCl, pH 7.5 and 10 mM MgCl₂. RNAs were renatured by cooling the reaction mixture to 37°C for 10-15 min. Cleavage reaction was initiated by mixing the labeled substrate RNA and total cellular RNA at 37°C. The reaction was allowed to proceed for ~ 18h, 25 following which the samples were resolved on a 20 % urea-polyacrylamide gel. Bands were visualized by autoradiography.

Figures 38 and 39. Ribozyme expression and activity levels in S35-transduced clonal CEM cell lines. 38) Northern analysis of S35-transduced clonal CEM cell lines. Standard curve was generated by 30 spiking known concentrations of in vitro transcribed S5 RNA into total cellular RNA isolated from non-transduced CEM cells. Pool, contains RNA from pooled cells transduced with S35 construct. Pool (-G418 for 3 Mo), contains RNA from pooled cells that were initially selected for resistance to G418 and then grown in the absence of G418 for 3 months. Lanes A through N contain RNA from individual clones that were generated from the 35 pooled cells transduced with S35 construct. tRNA_i^{met}, refers to the

endogenous tRNA. S35, refers to the position of the ribozyme band. M, marker lane. 39) Activity levels in S35-transduced clonal CEM cell lines. RNA isolation and cleavage reactions were as described in Fig.37. Nomenclature is same as in Figs. 35 and 36 except, S, 5' terminus-labeled 5 substrate RNA. P, 8 nt 5' terminus-labeled ribozyme-mediated RNA cleavage product.

Figures 40 and 41 are proposed secondary structures of S35 and S35 containing a desired RNA (HHI), respectively. The position of HHI ribozyme is indicated in figure 41. Intramolecular stem refers to the stem 10 structure formed due to an intramolecular base-paired interaction between the 3' sequence and the complementary 5' terminus. The length of the stem ranges from 15-16 base-pairs. Location of the A and the B boxes are shown.

Figures 42 and 43 are proposed secondary structures of S35 plus 15 and S35 plus containing HHI ribozyme.

Figures 44, 45, 46 and 47 are the nucleotide base sequences of S35, HHIS35, S35 Plus, and HHIS35 Plus respectively.

Figs. 48a-b is a general formula for pol III RNA of this invention.

Figure 49 is a diagrammatic representation of 5T construct. In this 20 construct the desired RNA is located 3' of the intramolecular stem.

Figures 50 and 51 contain proposed secondary structures of 5T construct alone and 5T construct containing a desired RNA (HHI ribozyme) respectively.

Figure 52 is a diagrammatic representation of TRZ-tRNA chimeras. 25 The site of desired RNA insertion is indicated.

Figure 53 shows the general structure of HHITRZ-A ribozyme chimera. A hammerhead ribozyme targeted to site I is inserted into the stem II region of TRZ-tRNA chimera.

Figure 54 shows the general structure of HPITRZ-A ribozyme chimera. 30 A hairpin ribozyme targeted to site I is cloned into the indicated region of TRZ-tRNA chimera.

Figure 55 shows a comparison of RNA cleavage activity of HHITRZ-A, HHITRZ-B and a chemically synthesized HHI hammerhead ribozymes.

Figure 56 shows expression of ribozymes in T cell lines that are stably transduced with viral vectors. M, markers; lane 1, non-transduced CEM 5 cells; lanes 2 and 3, MT2 and CEM cells transduced with retroviral vectors; lanes 4 and 5, MT2 and CEM cells transduced with AAV vectors.

Figs. 57a-b Schematic diagram of adeno-associated virus and adenoviruses vectors for ribozyme delivery. Both vectors utilize one or more ribozyme encoding transcription units (RZ) based on RNA polymerase II or 10 RNA polymerase III promoters. A. Diagram of an AAV-based vector containing minimal AAV sequences comprising the inverted terminal repeats (ITR) at each end of the vector genome, an optional selectable marker (Neo) driven by an exogenous promoter (Pro), a ribozyme transcription unit, and sufficient additional sequences (stuffer) to maintain a 15 vector length suitable for efficient packaging. B. Diagram of ribozyme expressing adenovirus vectors containing deletions of one or more wild type adenovirus coding regions (cross-hatched boxes marked as E1, pIX, E3, and E4), and insertion of the ribozyme transcription unit at any or several of those regions of deletions.

Fig. 58 is a graph showing the effect of arm length variation on the 20 activity of ligated hammerhead (HH) ribozymes. Nomenclature 5/5, 6/6, 7/7, 8/8 and so on refers to the number of base-pairs being formed between the ribozyme and the target. For example, 5/8 means that the HH ribozyme forms 5 bp on the 5' side and 8 bp on the 3' side of the cleavage site for a 25 total of 13 bp. $-\Delta G$ refers to the free energy of binding calculated for base-paired interactions between the ribozyme and the substrate RNA (Turner and Sugimoto, 1988 *Ann. Rev. Biophys. Chem.* 17, 167). RPI A is a HH ribozyme with 6/6 binding arms.

Figs. 59 and 60 and 61 show cleavage of long substrate (622 nt) by 30 ligated HH ribozymes.

Fig. 62 is a diagrammatic representation of a hammerhead ribozyme (HH-H) targeted against a site termed H. Variants of HH-H are also shown that contain either a 2 base-paired stem II (HH-H1 and HH-H2) or a 3 base-paired stem II (HH-H3 and HH-H4).

Figs. 63 and 64 show RNA cleavage activity of HH-I and its variants (see Fig.62). 63) cleavage of matched substrate RNA (15 nt). 64) cleavage of long substrate RNA (613 nt).

Figs. 65a-b is a schematic representation of a method of this invention
5 to synthesize a full length hairpin ribozyme. No splint strand is required for ligation but rather the two fragments hybridize together at helix 4 prior to ligation. The only prerequisite is that the 3' fragment is phosphorylated at its 5' end and that the 3' end of the 5' fragment have a hydroxyl group. The hairpin ribozyme is targeted against site J. H1 and H2 are intermolecular
10 H3 and H4 are intramolecular helices formed within the hairpin ribozyme motif. Arrow indicates the cleavage site.

Fig. 66 shows RNA cleavage activity of ligated hairpin ribozymes targeted against site J.

15 Figs. 67a-b is a diagrammatic representation of a Site K Hairpin Ribozyme (HP-K) showing the proposed secondary structure of the hairpin ribozyme •substrate complex as described in the art (Berzal-Herranz *et al.*, 1993 *EMBO J.* 12, 2567). The ribozyme has been assembled from two fragments (bimolecular ribozyme; Chowrima and Burke, 1992 *Nucleic Acids Res.* 20, 2835); #H1 and H2 represent intermolecular helix formation between the ribozyme and the substrate. H3 and H4 represent intramolecular helix formation within the ribozyme (intermolecular helix in the case of bimolecular ribozyme). Left panel (HP-K1) indicates 4 base-paired helix 2 and the right panel (HP-K2) indicates 6 base-paired helix 2.
20 25 Arrow indicates the site of RNA cleavage. All the ribozymes discussed herein were chemically synthesized by solid phase synthesis using RNA phosphoramidite chemistry, unless otherwise indicated. Those skilled in the art will recognize that these ribozymes could also be made transcriptionally *in vitro* and *in vivo*.

30 Figure 68 is a graph showing RNA cleavage by hairpin ribozymes targeted to site K. A plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-K2 (6 bp helix 2) cleaves a 422 target RNA to a greater extent than the HP-K1 (4 bp helix 2).

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 422 nt region (containing hairpin site A) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α -32P]CTP (Chowrira & Burke, 1991 *supra*). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1 μ M) and internally labeled 422 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Figs. 69a-b is the Site L Hairpin Ribozyme (HP-L) showing proposed secondary structure of the hairpin ribozyme-substrate complex. The ribozyme was assembled from two fragments as described above. The nomenclature is the same as above.

Figure 70 shows RNA cleavage by hairpin ribozymes targeted to site L. A plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-L2 (6 bp helix 2) cleaves a 2 KB target RNA to a greater extent than the HP-L1 (4 bp helix 2). To make internally-labeled substrate RNA for *trans*-ribozyme cleavage reactions, a 2 kB region (containing hairpin site L) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. The cleavage reactions were carried out as described above.

Figs. 71a-b shows a Site M Hairpin Ribozyme (HP-M) with the proposed secondary structure of the hairpin ribozyme•substrate complex. The ribozyme was assembled from two fragments as described above.

Figure 72 is a graph showing RNA cleavage by hairpin ribozymes targeted to site M. The ribozymes were tested at both 20°C and at 26°C. To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 1.9 KB region (containing hairpin site M) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Cleavage reactions were carried out as described above except that 20°C and at 26°C temperatures were used.

Figs. 73a-d shows various structural modifications of the present invention. A) Hairpin ribozyme lacking helix 5. Nomenclature is same as described under figure 3. B) Hairpin ribozyme lacking helix 4 and helix 5. Helix 4 is replaced by a nucleotide loop wherein $q \geq 2$ bases. C) Nomenclature is same as described under figure 3. D) Hairpin ribozyme lacking helix 5. Helix 4 loop is replaced by a linker 103'L", wherein L is a non-nucleotide linker molecule (Benseler *et al.*, 1993 *J. Am. Chem. Soc.* 115, 8483; Jennings *et al.*, WO 94/13688). Nomenclature is same as described under figure 3. E) Hairpin ribozyme lacking helix 4 and helix 5. Helix 4 is replaced by non-nucleotide linker molecule "L" (Benseler *et al.*, 1993 *supra*; Jennings *et al.*, *supra*). Nomenclature is same as described under figure 3.

Figs. 74a-b shows Hairpin ribozymes containing nucleotide spacer region "s" at the indicated location, wherein s is ≥ 1 base. Hairpin ribozymes containing spacer region, can be synthesized as one fragment or can be assembled from multiple fragments. Nomenclature is same as described under figure 3.

Figs. 75a-e shows the structures of the 5'-C-alkyl-modified nucleotides. R₁ is as defined above. R is OH, H, O-protecting group, NH, or any group described by the publications discussed above, and those described below. B is as defined in the Figure or any other equivalent nucleotide base. CE is cyanoethyl, DMT is a standard blocking group. Other abbreviations are standard in the art.

Figure 76 is a diagrammatic representation of the synthesis of 5'-C-alkyl-D-allose nucleosides and their phosphoramidites.

Figure 77 is a diagrammatic representation of the synthesis of 5'-C-alkyl-L-talose nucleosides and their phosphoramidites.

- 5 Figure 78 is a diagrammatic representation of hammerhead ribozymes targeted to site O containing 5'-C-methyl-L-talo modifications at various positions.

Figure 79 shows RNA cleavage activity of HH-O ribozymes. Fraction of target RNA uncleaved as a function of time is shown.

- 10 Figure 80 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al. Nucleic Acids Res.* 1992, 20, 3252) showing specific substitutions.

- 15 Figs. 81a-j shows the structures of various 2'-alkyl modified nucleotides which exemplify those of this invention. R groups are alkyl groups, Z is a protecting group.

Figure 82 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine.

Figure 83 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene uridine.

- 20 Figure 84 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene cytidine.

Figure 85 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene adenosine.

- 25 Figure 86 is a diagrammatic representation of the synthesis of 2'-C-carboxymethylidine uridine, 2'-C-methoxycarboxymethylidine uridine and derivatized amidites thereof. X is CH₃ or alkyl as discussed above, or another substituent.

Figure 87 is a diagrammatic representation of a synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonates.

Figure 88 is a diagrammatic representation of the synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonate 3'-phosphoramidites, dimers and solid supported dimers.

Figure 89 is a diagrammatic representation of the synthesis of 5 nucleoside 5'-deoxy-5'-difluoromethylene triphosphates.

Figures 90 and 91 are diagrammatic representations of the synthesis of 3'-deoxy-3'-difluoromethylphosphonates and dimers.

Figure 92 is a schematic representation of synthesizing RNA phosphoramidite of a nucleotide containing a 2'-hydroxyl group 10 modification of the present invention.

Figs. 93a-b describes a method for deprotection of oligonucleotides containing a 2'-hydroxyl group modification of the present invention.

Figure 94 is a diagrammatic representation of a hammerhead 15 ribozyme targeted to site N. Positions of 2'-hydroxyl group substitution is indicated.

Figure 95 shows RNA cleavage activity of ribozymes containing a 2'-hydroxyl group modification of the present invention. All RNA, represents hammerhead ribozyme (HHN) with no 2'-hydroxyl group modifications. U7-ala, represents HHN ribozyme containing 2'-NH-alanine modification at the 20 U7 position. U4/U7-ala, represents HHA containing 2'-NH-alanine modifications at U4 and U7 positions. U4 lys, represents HHA containing 2'-NH-lysine modification at U4 position. U7 lys, represents HHA containing 2'-NH-lysine modification at U7 position. U4/U7-lys, represents HHN containing 2'-NH-lysine modification at U4 and U7 positions.

25 Figures 96 and 97 are schematic representations of synthesizing (solid-phase synthesis) 3' ends of RNA with modification of the present invention. B, refers to either a base, modified base or an H.

Figure 98 and 99 are schematic representations of synthesizing (solid-phase synthesis) 5' ends of RNA with modification of the present 30 invention. B, refers to either a base, modified base or an H.

Figures 100 and 101 are general schematic representations of the invention.

Fig. 102a-d is a schematic representation of a method of the invention.

Fig. 103 is a graph of the results of the experiment diagrammed in figure 104.

Figure 104 is a diagrammatic representation of a fusion mRNA used
5 in the experiment diagrammed in Fig. 102.

Figure 105 is a diagrammatic representation of a method for selection of useful ribozymes of this invention.

Figure 106 generally shows R-loop formation, and an R-loop complex. In addition, it indicates the location at which ligands can be
10 provided to target the R-loop complex to cells using at least three different procedures, such as ligand receptor interaction, lipid or calcium phosphate mediated delivery, or electroporation.

Figure 107 shows a method for use of self-processing ribozymes to generate therapeutic ribozymes of unit length. This method is essentially
15 described by Draper et al., PCT WO 93/23509.

Figure 108 shows a method of linking ligands like folate, carbohydrate or peptides to R-loop forming RNA.

Ribozymes of this invention block to some extent ICAM-1, IL-5, rel A,
TNF- α , p210bcr-abl, or RSV genes expression and can be used to treat
20 diseases or diagnose such diseases. Ribozymes will be delivered to cells in culture and to tissues in animal models. Ribozyme cleavage of ICAM-1, IL-5, rel A, TNF- α , p210bcr-abl, or RSV mRNA in these systems may prevent or alleviate disease symptoms or conditions.

I. Target sites

Targets for useful ribozymes can be determined as disclosed in Draper et al PCT WO93/23509, Sullivan et al., PCT WO94/02595 as well as by Draper et al., PCT/US94/13129 and hereby incorporated by reference herein in totality. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested in vitro and in vivo, as also described. Such ribozymes can also be
25
30

optimized and delivered as described therein. While specific examples to animal and human RNA are provided, those in the art will recognize that the equivalent human RNA targets described can be used as described below. Thus, the same target may be used, but binding arms suitable for 5 targeting human RNA sequences are present in the ribozyme. Such targets may also be selected as described below.

It must be established that the sites predicted by the computer-based RNA folding algorithm correspond to potential cleavage sites. Hammerhead or hairpin ribozymes are designed that could bind and are 10 individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci., USA, 86 7706-7710) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm 15 lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

mRNA is screened for accessible cleavage sites by the method described generally in Draper et al., PCT WO93/23569 hereby incorporated by reference herein. Briefly, DNA oligonucleotides 20 representing potential hammerhead or hairpin ribozyme cleavage sites are synthesized. A polymerase chain reaction is used to generate a substrate for T7 RNA polymerase transcription from cDNA clones. Labeled RNA transcripts are synthesized *in vitro* from DNA templates. The oligonucleotides and the labeled transcripts are annealed, RNaseH is 25 added and the mixtures are incubated for the designated times at 37°C. Reactions are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a phosphor imaging system. From these data, hammerhead or hairpin ribozyme sites are chosen as the 30 most accessible.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used 35 follows the procedure for normal RNA synthesis as described in Usman et al., 1987 *J. Am. Chem. Soc.*, 109, 7845 and in Scaringe et al., 1990

Nucleic Acids Res., 18, 5433 and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, phosphoramidites at the 3'-end. The average stepwise coupling yeilds are >98%. Inactive ribozymes are synthesized by substituting a U for G5 and a 5 U for A14 (numbering from Hertel et al., 1992 *Nucleic Acids Res.*, 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840). Ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and 10 Uhlenbach, 1989, *Methods Enzymol.*, 180, 51). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'H (for a review see Usman and Cedergren, 1992 *TIBS* 17,34). Ribozymes are purified by gel electrophoresis using heneral methods or are purified by 15 high pressure liquid chromatography and are resuspended in water.

Example 1: ICAM-1

Ribozymes that cleave ICAM-1 mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders. ICAM-1 function can be blocked therapeutically using monoclonal antibodies. Ribozymes have 20 the advantage of being generally immunologically inert, whereas significant neutralizing anti-IgG responses can be observed with some monoclonal antibody treatments.

The following is a brief description of the physiological role of ICAM-1. The discussion is not meant to be complete and is provided only for 25 understanding of the invention that follows. This summary is not an admission that any of the work described below is prior art to the claimed invention.

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface protein whose expression is induced by inflammatory mediators. ICAM-1 is required for adhesion of leukocytes to endothelial cells and for several 30 immunological functions including antigen presentation, immunoglobulin production and cytotoxic cell activity. Blocking ICAM-1 function prevents immune cell recognition and activity during transplant rejection and in animal models of rheumatoid arthritis, asthma and reperfusion injury.

- Cell-cell adhesion plays a pivotal role in inflammatory and immune responses (Springer et al., 1987 *Ann. Rev. Immunol.* 5, 223-252). Cell adhesion is required for leukocytes to bind to and migrate through vascular endothelial cells. In addition, cell-cell adhesion is required for antigen presentation to T cells, for B cell induction by T cells, as well as for the cytotoxicity activity of T cells, NK cells, monocytes or granulocytes. Intercellular adhesion molecule-1 (ICAM-1) is a 110 kilodalton member of the immunoglobulin superfamily that is involved in all of these cell-cell interactions (Simmons et al., 1988 *Nature (London)* 331, 624-627).
- 5 ICAM-1 is expressed on only a limited number of cells and at low levels in the absence of stimulation (Dustin et al., 1986 *J. Immunol.* 137, 245-254). Upon treatment with a number of inflammatory mediators (lipopolysaccharide, γ -interferon, tumor necrosis factor- α , or interleukin-1), a variety of cell types (endothelial, epithelial, fibroblastic and hematopoietic 10 cells) in a variety of tissues express high levels of ICAM-1 on their surface (Sringer et. al. *supra*; Dustin et al., *supra*; and Rothlein et al., 1988 *J. Immunol.* 141, 1665-1669). Induction occurs via increased transcription of ICAM-1 mRNA (Simmons et al., *supra*). Elevated expression is detectable after 4 hours and peaks after 16 - 24 hours of induction.
- 15 ICAM-1 induction is critical for a number of inflammatory and immune responses. *In vitro*, antibodies to ICAM-1 block adhesion of leukocytes to cytokine-activated endothelial cells (Boyd, 1988 *Proc. Natl. Acad. Sci. USA* 85, 3095-3099; Dustin and Springer, 1988 *J. Cell Biol.* 107, 321-331). Thus, ICAM-1 expression may be required for the extravasation of immune 20 cells to sites of inflammation. Antibodies to ICAM-1 also block T cell killing, mixed lymphocyte reactions, and T cell-mediated B cell differentiation, suggesting that ICAM-1 is required for these cognate cell interactions (Boyd et al., *supra*). The importance of ICAM-1 in antigen presentation is underscored by the inability of ICAM-1 defective murine B cell mutants to 25 stimulate antigen-dependent T cell proliferation (Dang et al., 1990 *J. Immunol.* 144, 4082-4091). Conversely, murine L cells require transfection with human ICAM-1 in addition to HLA-DR in order to present antigen to human T cells (Altmann et al., 1989 *Nature (London)* 338, 512-514). In summary, evidence *in vitro* indicates that ICAM-1 is required for cell-cell 30 interactions critical to inflammatory responses, cellular immune responses, and humoral antibody responses.
- 35

By engineering ribozyme motifs we have designed several ribozymes directed against ICAM-1 mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance. These ribozymes cleave ICAM-1 target sequences *in vitro*.

5 The sequence of human, rat and mouse ICAM-1 mRNA can be screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Tables 2, 3, and 6-9. (All sequences
10 are 5' to 3' in the tables) While rat, mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility.

15 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 4 - 8 and 10. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

20 The ribozymes will be tested for function *in vivo* by exogenous delivery to human umbilical vein endothelial cells (HUVEC). Ribozymes will be delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors described above. Cytokine-induced ICAM-1 expression will be
25 monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. ICAM-1 mRNA levels will be assessed by Northern, by RNase protection, by primer extension or by quantitative RT-PCR analysis. Ribozymes that block the induction of ICAM-1 protein and mRNA by more than 90% will be identified.

30 As disclosed by Sullivan et al., PCT WO94/02595, incorporated by reference herein, ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of ICAM-1 mRNA and protein. The effect of the anti-ICAM-1 ribozymes on graft
35 rejection will then be assessed. Similarly, ribozymes will be introduced

into joints of mice with collagen-induced arthritis or rabbits with *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-ICAM-1 ribozyme or a gene 5 construct that constitutively expresses the ribozyme may abrogate inflammatory and immune responses in these diseases.

Uses

ICAM-1 plays a central role in immune cell recognition and function. Ribozyme inhibition of ICAM-1 expression can reduce transplant rejection 10 and alleviate symptoms in patients with rheumatoid arthritis, asthma or other acute and chronic inflammatory disorders. We have engineered several ribozymes that cleave ICAM-1 mRNA. Ribozymes that efficiently inhibit ICAM-1 expression in cells can be readily found and their activity measured with regard to their ability to block transplant rejection and 15 arthritis symptoms in animal models. These anti-ICAM-1 ribozymes represent a novel therapeutic for the treatment of immunological or inflammatory disorders.

The therapeutic utility of reduction of activity of ICAM-1 function is evident in the following disease targets. The noted references indicate the 20 role of ICAM-1 and the therapeutic potential of ribozymes described herein. Thus, these targets can be therapeutically treated with agents that reduce ICAM-1 expression or function. These diseases and the studies that support a critical role for ICAM-1 in their pathology are listed below. This 25 list is not meant to be complete and those in the art will recognize further conditions and diseases that can be effectively treated using ribozymes of the present invention.

- Transplant rejection

ICAM-1 is expressed on venules and capillaries of human cardiac biopsies with histological evidence of graft rejection (Briscoe et al., 1991 *Transplantation* 30 51, 537-539).

Antibody to ICAM-1 blocks renal (Cosimi et al., 1990 *J. Immunol.* 144, 4604-4612) and cardiac (Flavin et al., 1991 *Transplant. Proc.* 23, 533-534) graft rejection in primates.

A Phase I clinical trial of a monoclonal anti-ICAM-1 antibody showed significant reduction in rejection and a significant increase in graft function in human kidney transplant patients (Haug, et al., 1993 *Transplantation* 55, 766-72).

- Rheumatoid arthritis

5 ICAM-1 overexpression is seen on synovial fibroblasts, endothelial cells, macrophages, and some lymphocytes (Chin et al., 1990 *Arthritis Rheum* 33, 1776-86; Koch et al., 1991 *Lab Invest* 64, 313-20).

Soluble ICAM-1 levels correlate with disease severity (Mason et al., 1993 *Arthritis Rheum* 36, 519-27).

10 Anti-ICAM antibody inhibits collagen-induced arthritis in mice (Kakimoto et al., 1992 *Cell Immunol* 142, 326-37).

Anti-ICAM antibody inhibits adjuvant-induced arthritis in rats (Iigo et al., 1991 *J Immunol* 147, 4167-71).

- Myocardial ischemia, stroke, and reperfusion injury

15 Anti-ICAM-1 antibody blocks adherence of neutrophils to anoxic endothelial cells (Yoshida et al., 1992 *Am J Physiol* 262, H1891-8).

Anti-ICAM-1 antibody reduces neurological damage in a rabbit model of cerebral stroke (Bowes et al., 1993 *Exp Neurol* 119, 215-9).

20 Anti-ICAM-1 antibody protects against reperfusion injury in a cat model of myocardial ischemia (Ma et al., 1992 *Circulation* 86, 937-46).

- Asthma

Antibody to ICAM-1 partially blocks eosinophil adhesion to endothelial cells and is overexpressed on inflamed airway endothelium and epithelium *in vivo* (Wegner et al., 1990 *Science* 247, 456-9).

25 In a primate model of asthma, anti-ICAM-1 antibody blocks airway eosinophilia (Wegner et al., *supra*) and prevents the resurgence of airway inflammation and hyper-responsiveness after dexamethasone treatment (Gundel et al., 1992 *Clin Exp Allergy* 22, 569-75).

- Psoriasis

Surface ICAM-1 and a clipped, soluble version of ICAM-1 is expressed in psoriatic lesions and expression correlates with inflammation (Kellner et al., 1991 *Br J Dermatol* 125, 211-6; Griffiths 1989 *J Am Acad Dermatol* 20, 617-29; Schopf et al., 1993 *Br J Dermatol* 128, 34-7).

- 5 Anti-ICAM antibody blocks keratinocyte antigen presentation to T cells (Nickoloff et al., 1993 *J Immunol* 150, 2148-59).

- Kawasaki disease

Surface ICAM-1 expression correlates with the disease and is reduced by effective immunoglobulin treatment (Leung, et al., 1989 *Lancet* 2, 1298-302).

- 10 Soluble ICAM levels are elevated in Kawasaki disease patients; particularly high levels are observed in patients with coronary artery lesions (Furukawa et al., 1992 *Arthritis Rheum* 35, 672-7; Tsuji, 1992 *Arerugi* 41, 1507-14).

- 15 Circulating LFA-1+ T cells are depleted (presumably due to ICAM-1 mediated extravasation) in Kawasaki disease patients (Furukawa et al., 1993 *Scand J Immunol* 37, 377-80).

Example 2: IL-5

- 20 Ribozymes that cleave IL-5 mRNA represent a novel therapeutic approach to inflammatory disorders like asthma. The invention features use of ribozymes to treat chronic asthma, e.g., by inhibiting the synthesis of IL-5 in lymphocytes and preventing the recruitment and activation of eosinophils.

- 25 A number of cytokines besides IL-5 may also be involved in the activation of inflammation in asthmatic patients, including platelet activating factor, IL-1, IL-3, IL-4, GM-CSF, TNF- α , gamma interferon, VCAM, ILAM-1, ELAM-1 and NF- κ B. In addition to these molecules, it is appreciated that any cellular receptors which mediate the activities of the cytokines are also good targets for intervention in inflammatory diseases. These targets include, but are not limited to, the IL-1R and TNF- α R on keratinocytes, epithelial and endothelial cells in airways. Recent data suggest that certain
30 neuropeptides may play a role in asthmatic symptoms. These peptides include substance P, neurokinin A and calcitonin-gene-related peptides. These target genes may have more general roles in inflammatory diseases, but are currently assumed to have a role only in asthma.

Ribozymes of this invention block to some extent IL-5 expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of asthma (Clutterbuck et al., 1989 *supra*; Garssen et al., 1991 *Am. Rev. Respir. Dis.* 144, 931-938; Larsen et al., 1992 *J. Clin. Invest.* 89, 747-752; Mauser et al., 1993 *supra*). Ribozyme cleavage of IL-5 mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse IL-5 mRNA were screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 11, 13, and 14, 15. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 12, lower case letters indicate positions that are not conserved between the Human and the Mouse IL-5 sequences.)

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 12, 14 - 16. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem loop II sequence of hammerhead ribozymes listed in Tables 12 and 14 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 15 and 16 (5'-CACGUUGUG-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 12, 14 - 16 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against IL-5 mRNA sequences. These ribozymes are synthesized

with modifications that improve their nuclease resistance. The ability of ribozymes to cleave IL-5 target sequences *in vitro* is evaluated.

- The ribozymes will be tested for function *in vivo* by analyzing IL-5 expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors. IL-5 expression will be monitored by biological assays, ELISA, by indirect immunofluorescence, and/or by FACS analysis. IL-5 mRNA levels will be assessed by Northern analysis, RNase protection or primer extension analysis or quantitative RT-PCR.
- 5 Ribozymes that block the induction of IL-5 activity and/or IL-5 mRNA by more than 90% will be identified.
- 10

Uses

Interleukin 5 (IL-5), a cytokine produced by CD4+ T helper cells and mast cells, was originally termed B cell growth factor II (reviewed by 15 Takatsu et al., 1988 *Immunol. Rev.* 102, 107). It stimulates proliferation of activated B cells and induces production of IgM and IgA. IL-5 plays a major role in eosinophil function by promoting differentiation (Clutterbuck et al., 1989 *Blood* 73, 1504-12), vascular adhesion (Walsh et al., 1990 *Immunology* 71, 258-65) and *in vitro* survival of eosinophils (Lopez et al., 20 1988 *J. Exp. Med.* 167, 219-24). This cytokine also enhances histamine release from basophils (Hirai et al., 1990 *J. Exp. Med.* 172, 1525-8). The following summaries of clinical results support the selection of IL-5 as a primary target for the treatment of asthma:

Several studies have shown a direct correlation between the number 25 of activated T cells and the number of eosinophils from asthmatic patients vs. normal patients (Oehling et al., 1992 *J. Investig. Allergol. Clin. Immunol.* 2, 295-9). Patients with either allergic asthma or intrinsic asthma were treated with corticosteroids. The bronchoalveolar lavage was monitored for 30 eosinophils, activated T helper cells and recovery of pulmonary function over a 28 to 30 day period. The number of eosinophils and activated T helper cells decreased progressively with subsequent improvement in pulmonary function compared to intrinsic asthma patients with no corticosteroid treatment.

Bronchoalveolar lavage cells were screened for production of 35 cytokines using *in situ* hybridization for mRNA. *In situ* hybridization signals

were detected for IL-2, IL-3, IL-4, IL-5 and GM-CSF. Upregulation of mRNA was observed for IL-4, IL-5 and GM-CSF (Robinson et al., 1993 J. Allergy Clin. Immunol. 92, 313-24). Another study showed that upregulation of IL-5 transcripts from allergen challenged vs. saline challenged asthmatic
5 patients (Krishnaswamy et al., 1993 Am. J. Respir. Cell. Mol. Biol. 9, 279-86).

An 18 patient study was performed to determine a mechanism of action for corticosteroid improvement of asthma symptoms. Improvement was monitored by methacholine responsiveness. A correlation was
10 observed between the methacholine responsiveness, a reduction in the number of eosinophils, a reduction in the number of cells expressing IL-4 and IL-5 mRNA and an increase in number of cells expressing interferon-gamma.

Bronchial biopsies from 15 patients were analyzed 24 hours after
15 allergen challenge (Bentley et al., 1993 Am. J. Respir. Cell. Mol. Biol. 8, 35-42). Increased numbers of eosinophils and IL-2 receptor positive cells were found in the biopsies. No differences in the numbers of total leukocytes, T lymphocytes, elastase-positive neutrophils, macrophages or mast cell subtypes were observed. The number of cells expressing IL-5
20 and GM-CSF mRNA significantly increased.

In another patient study, the eosinophil phenotype was the same for asthmatic patients and normal individuals. However, eosinophils from asthmatic patients had greater leukotriene C4 producing capacity and migration capacity. There were elevated levels of IL-3, IL-5 and GM-CSF in
25 the circulation of asthmatics but not in normal individuals (Bruijnzeel et al., 1992 Schweiz. Med. Wochenschr. 122, 298-301).

Efficacy of antibody to IL-5 was assessed in a guinea pig asthma model. The animals were challenged with ovalbumin and assayed for eosinophilia and the responsiveness to the bronchoconstriction substance
30 P. A 30 mg/kg dose of antibody administered i.p. blocked ovalbumin-induced increased sensitivity to substance P and blocked increases in bronchoalveolar and lung tissue accumulation of eosinophils (Mauser et al., 1993 Am. Rev. Respir. Dis. 148, 1623-7). In a separate study guinea pigs challenged for eight days with ovalbumin were treated with
35 monoclonal antibody to IL-5. Treatment produced a reduction in the

number of eosinophils in bronchoalveolar lavage. No reduction was observed for unchallenged guinea pigs and guinea pigs treated with a control antibody. Antibody treatment completely inhibited the development of hyperreactivity to histamine and arecoline after ovalbumin challenge
5 (van Oosterhout et al., 1993 Am. Rev. Respir. Dis. 147, 548-52)

Results obtained from human clinical analysis and animal studies indicate the role of activated T helper cells, cytokines and eosinophils in asthma. The role of IL-5 in eosinophil development and function makes IL-
10 5 a good candidate for target selection. The antibody studies neutralized IL-5 in the circulation thus preventing eosinophilia. Inhibition of the production of IL-5 will achieve the same goal.

Asthma – a prominent feature of asthma is the infiltration of eosinophils and deposition of toxic eosinophil proteins (e.g. major basic protein, eosinophil-derived neurotoxin) in the lung. A number of T-cell-derived factors like IL-5 are responsible for the activation and maintenance of eosinophils (Kay, 1991 J. Allergy Clin. Immun. 87, 893). Inhibition of IL-5 expression in the lungs can decrease the activation of eosinophils and will help alleviate the symptoms of asthma.
15

Atopy – is characterized by the development of type I hypersensitive reactions associated with exposure to certain environmental antigens. One of the common clinical manifestations of atopy is eosinophilia (accumulation of abnormally high levels of eosinophils in the blood). Antibodies against IL-5 have been shown to lower the levels of eosinophils in mice (Cook et al., 1993 in Immunopharmacol. Eosinophils ed. Smith and
25 Cook, pp. 193-216, Academic, London, UK)

Parasitic infection-related eosinophilia– infections with parasites like helminths, can lead to severe eosinophilia (Cook et al., 1993 supra). Animal models for eosinophilia suggest that infection of mice, for example, can lead to blood, peritoneal and/or tissue eosinophilia, all of
30 which seem to be lowered to varying degrees by antibodies directed against IL-5.

Pulmonary infiltration eosinophilia– is characterised by accumulation of high levels of eosinophils in pulmonary parenchyma (Gleich, 1990 J. Allergy Clin. Immunol. 85, 422).

L-Tryptophan-associated eosinophilia-myalgia syndrome (EMS)-

The EMS disease is closely linked to the consumption of L-tryptophan, an essential aminoacid used to treat conditions like insomnia (for review see Varga et al., 1993 *J Invest. Dermatol.* 100, 97s). Pathologic

5 and histologic studies have demonstrated high levels of eosinophils and mononuclear inflammatory cells in patients with EMS. It appears that IL-5 and transforming growth factor play a significant role in the development of EMS (Varga et al., 1993 *supra*) by activating eosinophils and other inflammatory cells.

10 Thus, ribozymes of the present invention that cleave IL-5 mRNA and thereby IL-5 activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits IL-5 function is described above; available cellular and activity assays are
15 numerous, reproducible, and accurate. Animal models for IL-5 function and for each of the suggested disease targets exist (Cook et al., 1993 *supra*) and can be used to optimize activity.

Example 3: NF- κ B

Ribozymes that cleave *rel A* mRNA represent a novel therapeutic
20 approach to inflammatory or autoimmune disorders. Inflammatory mediators such as lipopolysaccharide (LPS), interleukin-1 (IL-1) or tumor necrosis factor-a (TNF- α) act on cells by inducing transcription of a number of secondary mediators, including other cytokines and adhesion molecules. In many cases, this gene activation is known to be mediated by
25 the transcriptional regulator, NF- κ B. One subunit of NF- κ B, the *relA* gene product (termed RelA or p65) is implicated specifically in the induction of inflammatory responses. Ribozyme therapy, due to its exquisite specificity, is particularly well-suited to target intracellular factors that contribute to disease pathology. Thus, ribozymes that cleave mRNA encoded by *rel A* or
30 TNF- α may represent novel therapeutics for the treatment of inflammatory and autoimmune disorders.

The nuclear DNA-binding activity, NF- κ B, was first identified as a factor that binds and activates the immunoglobulin κ light chain enhancer in B cells. NF- κ B now is known to activate transcription of a variety of other cellular genes (e.g., cytokines, adhesion proteins, oncogenes and viral

proteins) in response to a variety of stimuli (e.g., phorbol esters, mitogens, cytokines and oxidative stress). In addition, molecular and biochemical characterization of NF- κ B has shown that the activity is due to a homodimer or heterodimer of a family of DNA binding subunits. Each 5 subunit bears a stretch of 300 amino acids that is homologous to the oncogene, *v-rel*. The activity first described as NF- κ B is a heterodimer of p49 or p50 with p65. The p49 and p50 subunits of NF- κ B (encoded by the *nf- κ B2* or *nf- κ B1* genes, respectively) are generated from the precursors NF- κ B1 (p105) or NF- κ B2 (p100). The p65 subunit of NF- κ B (now 10 termed Rel A) is encoded by the *rel A* locus.

The roles of each specific transcription-activating complex now are being elucidated in cells (N.D. Perkins, et al., 1992 Proc. Natl Acad. Sci. USA 89, 1529-1533). For instance, the heterodimer of NF- κ B1 and Rel A (p50/p65) activates transcription of the promoter for the adhesion molecule, 15 VCAM-1, while NF- κ B2/RelA heterodimers (p49/p65) actually inhibit transcription (H.B. Shu, et al., Mol. Cell. Biol. 13, 6283-6289 (1993)). Conversely, heterodimers of NF- κ B2/RelA (p49/p65) act with Tat-I to activate transcription of the HIV genome, while NF- κ B1/RelA (p50/p65) heterodimers have little effect (J. Liu, N.D. Perkins, R.M. Schmid, G.J. 20 Nabel, J. Virol. 1992 66, 3883-3887). Similarly, blocking *rel A* gene expression with antisense oligonucleotides specifically blocks embryonic stem cell adhesion; blocking NF- κ B1 gene expression with antisense oligonucleotides had no effect on cellular adhesion (Narayanan et al., 1993 Mol. Cell. Biol. 13, 3802-3810). Thus, the promiscuous role initially 25 assigned to NF- κ B in transcriptional activation (M.J. Lenardo, D. Baltimore, 1989 Cell 58, 227-229) represents the sum of the activities of the *rel* family of DNA-binding proteins. This conclusion is supported by recent transgenic "knock-out" mice of individual members of the *rel* family. Such "knock-outs" show few developmental defects, suggesting that essential 30 transcriptional activation functions can be performed by more than one member of the *rel* family.

A number of specific inhibitors of NF- κ B function in cells exist, including treatment with phosphorothioate antisense oligonucleotide, treatment with double-stranded NF- κ B binding sites, and over expression 35 of the natural inhibitor MAD-3 (an I κ B family member). These agents have

been used to show that NF- κ B is required for induction of a number of molecules involved in inflammation, as described below.

•NF- κ B is required for phorbol ester-mediated induction of IL-6 (I. Kitajima, et al., *Science* 258, 1792-5 (1992)) and IL-8 (Kunsch and Rosen, 5 *Mol. Cell. Biol.* 13, 6137-46).

•NF- κ B is required for induction of the adhesion molecules ICAM-1 (Eck, et al., 1993 *Mol. Cell. Biol.* 13, 6530-6536), VCAM-1 (Shu et al., *supra*), and E-selectin (Read, et al., 1994 *J. Exp. Med.* 179, 503-512) on endothelial cells.

10 •NF- κ B is involved in the induction of the integrin subunit, CD18, and other adhesive properties of leukocytes (Eck et al., 1993 *supra*).

The above studies suggest that NF- κ B is integrally involved in the induction of cytokines and adhesion molecules by inflammatory mediators. Two recent papers point to another connection between NF- κ B and inflammation: glucocorticoids may exert their anti-inflammatory effects by inhibiting NF- κ B. The glucocorticoid receptor and p65 both act at NF- κ B binding sites in the ICAM-1 promoter (van de Stolpe, et al., 1994 *J. Biol. Chem.* 269, 6185-6192). Glucocorticoid receptor inhibits NF- κ B-mediated induction of IL-6 (Ray and Prefontaine, 1994 *Proc. Natl Acad. Sci USA* 91, 15 752-756). Conversely, overexpression of p65 inhibits glucocorticoid induction of the mouse mammary tumor virus promoter. Finally, protein cross-linking and co-immunoprecipitation experiments demonstrated direct physical interaction between p65 and the glucocorticoid receptor (*Id.*). 20

25 Ribozymes of this invention block to some extent NF- κ B expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of restenosis, transplant rejection and rheumatoid arthritis. Ribozyme cleavage of *relA* mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

30 The sequence of human and mouse *relA* mRNA can be screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 17, 18 and 21-22. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and

ribozymes thereafter designed, the human targetted sequences are of most utility.

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 19 - 22. Those in the art will recognize that 5 these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

10 By engineering ribozyme motifs we have designed several ribozymes directed against *relA* mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave *relA* target sequences *in vitro* is evaluated.

15 The ribozymes will be tested for function *in vivo* by analyzing cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA and RNA vectors. Cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression will be monitored by ELISA, by indirect immunofluorescence, and/or by FACS 20 analysis. *Rel A* mRNA levels will be assessed by Northern analysis, RNase protection or primer extension analysis or quantitative RT-PCR. Activity of NF- κ B will be monitored by gel-retardation assays. Ribozymes that block the induction of NF- κ B activity and/or *rel A* mRNA by more than 50% will be identified.

25 RNA ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of VCAM-1, ICAM-1, IL-6 and IL-8 mRNA and protein. The effect of the anti-*rel A* ribozymes on graft rejection will then be assessed. Similarly, ribozymes will be introduced into joints of mice with collagen-induced arthritis or rabbits with *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-*relA* ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate 30 inflammatory and immune responses in these diseases.

Uses

A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves *rel A* mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

- 10 •Rheumatoid arthritis (RA).

Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the synovium: high efficiency of gene transfer and expression for several months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, *J. Clin. Invest.* 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple administrations may be necessary. Retrovirus and adeno-associated virus vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

•Restenosis.

25 Expression of NF- κ B in the vessel wall of pigs causes a narrowing of the luminal space due to excessive deposition of extracellular matrix components. This phenotype is similar to matrix deposition that occurs subsequent to coronary angioplasty. In addition, NF- κ B is required for the expression of the oncogene c-myb (F.A. La Rosa, J.W. Pierce, G.E. Soneneshein, *Mol. Cell. Biol.* 14, 1039-44 (1994)). Thus NF- κ B induces smooth muscle proliferation and the expression of excess matrix components: both processes are thought to contribute to reocclusion of vessels after coronary angioplasty.

•Transplantation.

NF- κ B is required for the induction of adhesion molecules (Eck et al., *supra*, K. O'Brien, et al., J. Clin. Invest. 92, 945-951 (1993)) that function in immune recognition and inflammatory responses. At least two potential modes of treatment are possible. In the first, transplanted organs are
5 treated *ex vivo* with ribozymes or ribozyme expression vectors. Transient inhibition of NF- κ B in the transplanted endothelium may be sufficient to prevent transplant-associated vasculitis and may significantly modulate graft rejection. In the second, donor B cells are treated *ex vivo* with ribozymes or ribozyme expression vectors. Recipients would receive the
10 treatment prior to transplant. Treatment of a recipient with B cells that do not express T cell co-stimulatory molecules (such as ICAM-1, VCAM-1, and/or B7 and B7-2) can induce antigen-specific anergy. Tolerance to the donor's histocompatibility antigens could result; potentially, any donor could be used for any transplantation procedure.

15 •Asthma.

Granulocyte macrophage colony stimulating factor (GM-CSF) is thought to play a major role in recruitment of eosinophils and other inflammatory cells during the late phase reaction to asthmatic trauma. Again, blocking the local induction of GM-CSF and other inflammatory
20 mediators is likely to reduce the persistent inflammation observed in chronic asthmatics. Aerosol delivery of ribozymes or adenovirus ribozyme expression vectors is a feasible treatment.

•Gene Therapy.

Immune responses limit the efficacy of many gene transfer
25 techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette that modulates inflammatory and immune responses into existing
30 adenovirus or retrovirus constructs will greatly enhance their potential.

Thus, ribozymes of the present invention that cleave *rel A* mRNA and thereby NF- κ B activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits NF- κ B

function is described above; available cellular and activity assays are number, reproducible, and accurate. Animal models for NF- κ B function (Kitajima, et al., *supra*) and for each of the suggested disease targets exist and can be used to optimize activity.

5 Example 4: TNF- α

Ribozymes that cleave the specific sites in TNF- α mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders.

Tumor necrosis factor- α (TNF- α) is a protein, secreted by activated leukocytes, that is a potent mediator of inflammatory reactions. Injection of
10 TNF- α into experimental animals can simulate the symptoms of systemic and local inflammatory diseases such as septic shock or rheumatoid arthritis.

TNF- α was initially described as a factor secreted by activated macrophages which mediates the destruction of solid tumors in mice (Old,
15 1985 Science 230, 4225-4231). TNF- α subsequently was found to be identical to cachectin, an agent responsible for the weight loss and wasting syndrome associated with tumors and chronic infections (Beutler, et al., 1985 Nature 316, 552-554). The cDNA and the genomic locus for TNF- α have been cloned and found to be related to TNF- β (Shakhov et al., 1990
20 J. Exp. Med. 171, 35-47). Both TNF- α and TNF- β bind to the same receptors and have nearly identical biological activities. The two TNF receptors have been found on most cell types examined (Smith, et al., 1990 Science 248, 1019-1023). TNF- α secretion has been detected from monocytes/macrophages, CD4+ and CD8+ T-cells, B-cells, lymphokine
25 activated killer cells, neutrophils, astrocytes, endothelial cells, smooth muscle cells, as well as various non-hematopoietic tumor cell lines (for a review see Turetskaya et al., 1991 in Tumor Necrosis Factor: Structure, Function, and Mechanism of Action B. B. Aggarwal, J. Vilcek, Eds. Marcel Dekker, Inc., pp. 35-60). TNF- α is regulated transcriptionally and translationally, and requires proteolytic processing at the plasma membrane in order to be secreted (Kriegler et al., 1988 Cell 53, 45-53). Once secreted, the serum half life of TNF- α is approximately 30 minutes. The tight regulation of TNF- α is important due to the extreme toxicity of this cytokine. Increasing evidence indicates that overproduction of TNF- α

during infections can lead to severe systemic toxicity and death (Tracey & Cerami, 1992 Am. J. Trop. Med. Hyg. 47, 2-7).

Antisense RNA and Hammerhead ribozymes have been used in an attempt to lower the expression level of TNF- α by targeting specified cleavage sites [Sioud et al., 1992 J. Mol. Biol. 223; 831; Sioud WO 94/10301; Kisich and co-workers, 1990 abstract (FASEB J. 4, A1860; 1991 slide presentation (J. Leukocyte Biol. sup. 2, 70); December, 1992 poster presentation at Anti-HIV Therapeutics Conference in SanDiego, CA; and "Development of anti-TNF- α ribozymes for the control of TNF- α gene expression"- Kisich, Doctoral Dissertation, 1993 University of California, Davis] listing various TNF α targeted ribozymes.

Ribozymes of this invention block to some extent TNF- α expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of septic shock and rheumatoid arthritis. Ribozyme cleavage of TNF- α mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse TNF- α mRNA can be screened for accessible sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 23, 25, and 27 - 28. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 24, lower case letters indicate positions that are not conserved between the human and the mouse TNF- α sequences.)

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 24, 26 - 28. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Tables 24 and 26 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any

sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 27 and 28 (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of 5 two base-paired stem structure can form. The sequences listed in Tables 24, 26 - 28 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables or AAV.

In a preferred embodiment of the invention, a transcription unit 10 expressing a ribozyme that cleaves TNF- α RNA is inserted into a plasmid DNA vector or an adenovirus DNA viral vector or AAV or alpha virus or retrovirus vectors. Viral vectors have been used to transfer genes to the intact vasculature or to joints of live animals (Willard et al., 1992 Circulation, 86, I-473.; Nabel et al., 1990 Science, 249, 1285-1288) and 15 both vectors lead to transient gene expression. The adenovirus vector is delivered as recombinant adenoviral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant adenovirus particles are locally administered to the site of treatment, e.g., through the use of an 20 injection catheter, stent or infusion pump or are directly added to cells or tissues *ex vivo*.

In another preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves TNF- α RNA is inserted into a retrovirus vector for sustained expression of ribozyme(s).

25 By engineering ribozyme motifs we have designed several ribozymes directed against TNF- α mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave TNF- α target sequences *in vitro* is evaluated.

The ribozymes will be tested for function in cells by analyzing 30 bacterial lipopolysaccharide (LPS)-induced TNF- α expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. TNF- α expression will be monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. TNF- α mRNA levels will be 35 assessed by Northern analysis, RNase protection, primer extension

analysis or quantitative RT-PCR. Ribozymes that block the induction of TNF- α activity and/or TNF- α mRNA by more than 90% will be identified.

RNA ribozymes and/or genes encoding them will be locally delivered to macrophages by intraperitoneal injection. After a period of ribozyme uptake, the peritoneal macrophages are harvested and induced *ex vivo* with LPS. The ribozymes that significantly reduce TNF- α secretion are selected. The TNF- α can also be induced after ribozyme treatment with fixed *Streptococcus* in the peritoneal cavity instead of *ex vivo*. In this fashion the ability of TNF- α ribozymes to block TNF- α secretion in a localized inflammatory response are evaluated. In addition, we will determine if the ribozymes can block an ongoing inflammatory response by delivering the TNF- α ribozymes after induction by the injection of fixed *Streptococcus*.

To examine the effect of anti-TNF- α ribozymes on systemic inflammation, the ribozymes are delivered by intravenous injection. The ability of the ribozymes to inhibit TNF- α secretion and lethal shock caused by systemic LPS administration are assessed. Similarly, TNF- α ribozymes can be introduced into the joints of mice with collagen-induced arthritis. Either free delivery, liposome delivery, cationic lipid delivery, adeno-associated virus vector delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model experiments can be used to supply ribozymes. One dose (or a few infrequent doses) of a stable anti-TNF- α ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate tissue damage in these inflammatory diseases.

Macrophage isolation.

To produce responsive macrophages 1 ml of sterile fluid thioglycollate broth (Difco, Detroit, MI.) was injected i.p. into 6 week old female C57bl/6NCR mice 3 days before peritoneal lavage. Mice were maintained as specific pathogen free in autoclaved cages in a laminar flow hood and given sterilized water to minimize "spontaneous" activation of macrophages. The resulting peritoneal exudate cells (PEC) were obtained by lavage using Hanks balanced salt solution (HBSS) and were plated at 2.5X10⁵/well in 96 well plates (Costar, Cambridge, MA.) with Eagles minimal essential medium (EMEM) containing 10% heat inactivated fetal

bovine serum. After adhering for 2 hours the wells were washed to remove non-adherent cells. The resulting cultures were 97% macrophages as determined by morphology and staining for non-specific esterase.

Transfection of ribozymes into macrophages:

5 The ribozymes were diluted to 2X final concentration, mixed with an equal volume of 11nM lipofectamine (Life Technologies, Gaithersburg, MD.), and vortexed. 100 ml of lipid:ribozyme complex was then added directly to the cells, followed immediately by 10 ml fetal bovine serum. Three hours after ribozyme addition 100 ml of 1 mg/ml bacterial 10 lipopolysaccharide (LPS) was added to each well to stimulate TNF production.

Quantitation of TNF- α in mouse macrophages:

Supernatants were sampled at 0, 2, 4, 8, and 24 hours post LPS stimulation and stored at -70°C. Quantitation of TNF- α was done by a 15 specific ELISA. ELISA plates were coated with rabbit anti-mouse TNF- α serum at 1:1000 dilution (Genzyme) followed by blocking with milk proteins and incubation with TNF- α containing supernatants. TNF- α was then detected using a murine TNF- α specific hamster monoclonal antibody (Genzyme). The ELISA was developed with goat anti-hamster IgG coupled 20 to alkaline phosphatase.

Assessment of reagent toxicity:

Following ribozyme/lipid treatment of macrophages and harvesting of supernatants viability of the cells was assessed by incubation of the cells with 5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium 25 bromide (MTT). This compound is reduced by the mitochondrial dihydrogenases, the activity of which correlates well with cell viability. After 12 hours the absorbance of reduced MTT is measured at 585 nm.

Uses

30 The association between TNF- α and bacterial sepsis, rheumatoid arthritis, and autoimmune disease make TNF- α an attractive target for therapeutic intervention [Tracy & Cerami 1992 supra; Williams et al., 1992 Proc. Natl. Acad. Sci. USA 89, 9784-9788; Jacob, 1992 J. Autoimmun. 5 (Supp. A), 133-143].

Septic Shock

Septic shock is a complication of major surgery, bacterial infection, and polytrauma characterized by high fever, increased cardiac output, reduced blood pressure and a neutrophilic infiltrate into the lungs and other major organs. Current treatment options are limited to antibiotics to reduce the bacterial load and non-steroidal anti-inflammatories to reduce fever. Despite these treatments in the best intensive care settings, mortality from septic shock averages 50%, due primarily to multiple organ failure and disseminated vascular coagulation. Septic shock, with an incidence of 5 200,000 cases per year in the United States, is the major cause of death in intensive care units. In septic shock syndrome, tissue injury or bacterial products initiate massive immune activation, resulting in the secretion of 10 pro-inflammatory cytokines which are not normally detected in the serum, such as TNF- α , interleukin-1 β (IL-1 β), γ -interferon (IFN- γ), interleukin-6 (IL-6), and interleukin-8 (IL-8). Other non-cytokine mediators such as 15 leukotriene b4, prostaglandin E2, C3a and C3d also reach high levels (de Boer et al., 1992 Immunopharmacology 24, 135-148).

TNF- α is detected early in the course of septic shock in a large fraction of patients (de Boer et al., 1992 supra). In animal models, injection of TNF- α has been shown to induce shock-like symptoms similar to those induced by LPS injection (Beutler et al., 1985 Science 229, 869-871); in contrast, injection of IL-1 β , IL-6, or IL-8 does not induce shock. Injection of TNF- α also causes an elevation of IL-1 β , IL-6, IL-8, PgE₂, acute phase proteins, and TxA₂ in the serum of experimental animals (de Boer et al., 1992 20 25 supra). In animal models the lethal effects of LPS can be blocked by pre-administration of anti-TNF- α antibodies. The cumulative evidence indicates that TNF- α is a key player in the pathogenesis of septic shock, and therefore a good candidate for therapeutic intervention.

Rheumatoid Arthritis

30 Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints leading to bone destruction and loss of joint function. At the cellular level, autoreactive T- lymphocytes and monocytes are typically present, and the synoviocytes often have altered morphology and immunostaining patterns. RA joints have been shown to 35 contain elevated levels of TNF- α , IL-1 α and IL-1 β , IL-6, GM-CSF, and TGF-

β (Abney et al., 1991 Imm. Rev. 119, 105-123), some or all of which may contribute to the pathological course of the disease.

- Cells cultured from RA joints spontaneously secrete all of the pro-inflammatory cytokines detected *in vivo*. Addition of antisera against TNF- α to these cultures has been shown to reduce IL-1 α/β production by these cells to undetectable levels (Abney et al., 1991 Supra). Thus, TNF- α may directly induce the production of other cytokines in the RA joint. Addition of the anti-inflammatory cytokine, TGF- β , has no effect on cytokine secretion by RA cultures. Immunocytochemical studies of human RA surgical specimens clearly demonstrate the production of TNF- α , IL-1 α/β , and IL-6 from macrophages near the cartilage/pannus junction when the pannus is invading and overgrowing the cartilage (Chu et al., 1992 Br. J. Rheumatology 31, 653-661). GM-CSF was shown to be produced mainly by vascular endothelium in these samples. Both TNF- α and TGF- β have been shown to be fibroblast growth factors, and may contribute to the accumulation of scar tissue in the RA joint. TNF- α has also been shown to increase osteoclast activity and bone resorption, and may have a role in the bone erosion commonly found in the RA joint (Cooper et al., 1992 Clin. Exp. Immunol. 89, 244-250).
- Elimination of TNF- α from the rheumatic joint would be predicted to reduce overall inflammation by reducing induction of MHC class II, IL-1 α/β , IL-6, and GM-CSF, and reducing T-cell activation. Osteoclast activity might also fall, reducing the rate of bone erosion at the joint. Finally, elimination of TNF- α would be expected to reduce accumulation of scar tissue within the joint by removal of a fibroblast growth factor.

- Treatment with an anti-TNF- α antibody reduces joint swelling and the histological severity of collagen-induced arthritis in mice (Williams et al., 1992 Proc. Natl. Acad. Sci. USA 89, 9784-9788). In addition, a study of RA patients who have received i.v. infusions of anti-TNF- α monoclonal antibody reports a reduction in the number and severity of inflamed joints after treatment. The benefit of monoclonal antibody treatment in the long term may be limited by the expense and immunogenicity of the antibody.

Psoriasis

- Psoriasis is an inflammatory disorder of the skin characterized by keratinocyte hyperproliferation and immune cell infiltrate (Kupper, 1990 J.

Clin. Invest. 86, 1783-1789). It is a fairly common condition, affecting 1.5-2.0% of the population. The disorder ranges in severity from mild, with small flaky patches of skin, to severe, involving inflammation of the entire epidermis. The cellular infiltrate of psoriasis includes T-lymphocytes, 5 neutrophils, macrophages, and dermal dendrocytes. The majority of T-lymphocytes are activated CD4⁺ cells of the T_H-1 phenotype, although some CD8⁺ and CD4⁺/CD8⁻ are also present. B lymphocytes are typically not found in abundance in psoriatic plaques.

Numerous hypotheses have been offered as to the proximal cause of 10 psoriasis including auto-antibodies and auto-reactive T-cells, overproduction of growth factors, and genetic predisposition. Although there is evidence to support the involvement of each of these factors in psoriasis, they are neither mutually exclusive nor are any of them necessary and sufficient for the pathogenesis of psoriasis (Reeves, 1991 15 Semin. Dermatol. 10, 217).

The role of cytokines in the pathogenesis of psoriasis has been investigated. Among those cytokines found to be abnormally expressed were TGF- α , IL-1 α , IL-1 β , IL-1ra, IL-6, IL-8, IFN- γ , and TNF- α . In addition to abnormal cytokine production, elevated expression of ICAM-1, ELAM-1, 20 and VCAM has been observed (Reeves, 1991 supra). This cytokine profile is similar to that of normal wound healing, with the notable exception that cytokine levels subside upon healing. Keratinocytes themselves have recently been shown to be capable of secreting EGF, TGF- α , IL-6, and TNF- α , which could increase proliferation in an autocrine fashion (Oxholm 25 et al., 1991 APMIS 99, 58-64).

Nickoloff et al., 1993 (J Dermatol Sci. 6, 127-33) have proposed the following model for the initiation and maintenance of the psoriatic plaque:

Tissue damage induces the wound healing response in the skin. Keratinocytes secrete IL-1 α , IL-1 β , IL-6, IL-8, TNF- α . These factors 30 activate the endothelium of dermal capillaries, recruiting PMNs, macrophages, and T-cells into the wound site.

Dermal dendrocytes near the dermal/epidermal junction remain activated when they should return to a quiescent state, and subsequently secrete cytokines including TNF- α , IL-6, and IL-8. Cytokine expression, in

turn, maintains the activated state of the endothelium, allowing extravasation of additional immunocytes, and the activated state of the keratinocytes which secrete TGF- α and IL-8. Keratinocyte IL-8 recruits immunocytes from the dermis into the epidermis. During passage through

5 the dermis, T-cells encounter the activated dermal dendrocytes which efficiently activate the T_H -1 phenotype. The activated T-cells continue to migrate into the epidermis, where they are stimulated by keratinocyte-expressed ICAM-1 and MHC class II. IFN- γ secreted by the T-cells synergizes with the TNF- α from dermal dendrocytes to increase

10 keratinocyte proliferation and the levels of TGF- α , IL-8, and IL-6 production. IFN- γ also feeds back to the dermal dendrocyte, maintaining the activated phenotype and the inflammatory cycle.

Elevated serum titres of IL-6 increases synthesis of acute phase proteins including complement factors by the liver, and antibody production

15 by plasma cells. Increased complement and antibody levels increases the probability of autoimmune reactions.

Maintenance of the psoriatic plaque requires continued expression of all of these processes, but attractive points of therapeutic intervention are

20 TNF- α expression by the dermal dendrocyte to maintain activated endothelium and keratinocytes, and IFN- γ expression by T-cells to maintain activated dermal dendrocytes.

There are 3 million patients in the United States afflicted with psoriasis. The available treatments for psoriasis are corticosteroids. The most widely prescribed are TEMOVATE (clobetasol propionate), LIDEX

25 (fluocinonide), DIPROLENE (betamethasone propionate), PSORCON (diflorasone diacetate) and TRIAMCINOLONE formulated for topical application. The mechanism of action of corticosteroids is multifactorial. This is a palliative therapy because the underlying cause of the disease remains, and upon discontinuation of the treatment the disease returns.

30 Discontinuation of treatment is often prompted by the appearance of adverse effects such as atrophy, telangiectasias and purpura. Corticosteroids are not recommended for prolonged treatments or when treatment of large and/or inflamed areas is required. Alternative treatments include retinoids, such as etretinate, which has been approved for

35 treatment of severe, refractory psoriasis. Alternative retinoid-based treatments are in advanced clinical trials. Retinoids act by converting

keratinocytes to a differentiated state and restoration of normal skin development. Immunosuppressive drugs such as cyclosporine are also in the advanced stages of clinical trials. Due to the nonspecific mechanism of action of corticosteroids, retinoids and immunosuppressives, these 5 treatments exhibit severe side effects and should not be used for extended periods of time unless the condition is life-threatening or disabling. There is a need for a less toxic, effective therapeutic agent in psoriatic patients.

HIV and AIDS

The human immunodeficiency virus (HIV) causes several 10 fundamental changes in the human immune system from the time of infection until the development of full-blown acquired immunodeficiency syndrome (AIDS). These changes include a shift in the ratio of CD4+ to CD8+ T-cells, sustained elevation of IL-4 levels, episodic elevation of TNF- α and TNF- β levels, hypergammaglobulinemia, and lymphoma/leukemia 15 (Rosenberg & Fauci, 1990 Immun. Today 11, 176; Weiss 1993 Science 260, 1273). Many patients experience a unique tumor, Kaposi's sarcoma and/or unusual opportunistic infections (e.g. *Pneumocystis carinii*, cytomegalovirus, herpesviruses, hepatitis viruses, papilloma viruses, and tuberculosis). The immunological dysfunction of individuals with AIDS 20 suggests that some of the pathology may be due to cytokine dysregulation.

Levels of serum TNF- α and IL-6 are often found to be elevated in AIDS patients (Weiss, 1993 supra). In tissue culture, HIV infection of monocytes isolated from healthy individuals stimulates secretion of both TNF- α and IL-6. This response has been reproduced using purified gp120, 25 the viral coat protein responsible for binding to CD-4 (Buonaguro et al., 1992 J. Virol. 66, 7159). It has also been demonstrated that the viral gene regulator, Tat, can directly induce TNF transcription. The ability of HIV to directly stimulate secretion of TNF- α and IL-6 may be an adaptive mechanism of the virus. TNF- α has been shown to upregulate transcription 30 of the LTR of HIV, increasing the number of HIV-specific transcripts in infected cells. IL-6 enhances HIV production, but at a post-transcriptional level, apparently increasing the efficiency with which HIV transcripts are translated into protein. Thus, stimulation of TNF- α secretion by the HIV virus may promote infection of neighboring CD4+ cells both by enhancing 35 virus production from latently infected cells and by driving replication of the virus in newly infected cells.

- The role of TNF- α in HIV replication has been well established in tissue culture models of infection (Sher et al., 1992 Immun. Rev. 127, 183), suggesting that the mutual induction of HIV replication and TNF- α replication may create positive feedback *in vivo*. However, evidence for the presence of such positive feedback in infected patients is not abundant. TNF- α levels are found to be elevated in some, but not all patients tested. Children with AIDS who were given zidovudine had reduced levels of TNF- α compared to those not given zidovudine (Cremoni et al., 1993 AIDS 7, 128). This correlation lends support to the hypothesis that reduced viral replication is physiologically linked to TNF- α levels. Furthermore, recently it has been shown that the polyclonal B cell activation associated with HIV infection is due to membrane-bound TNF- α . Thus, levels of secreted TNF- α may not accurately reflect the contribution of this cytokine to AIDS pathogenesis.
- Chronic elevation of TNF- α has been shown to result in cachexia (Tracey et al., 1992 Am. J. Trop. Med. Hyg. 47, 2-7), increased autoimmune disease (Jacob, 1992 supra), lethargy, and immune suppression in animal models (Aderka et al., 1992 Isr. J. Med. Sci. 28, 126-130). The cachexia associated with AIDS may be associated with chronically elevated TNF- α frequently observed in AIDS patients. Similarly, TNF- α can stimulate the proliferation of spindle cells isolated from Kaposi's sarcoma lesions of AIDS patients (Barillari et al., 1992 J Immunol 149, 3727).
- A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves the specified sites in TNF- α mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

*Septic shock.

Exogenous delivery of ribozymes to macrophages can be achieved by intraperitoneal or intravenous injections. Ribozymes will be delivered by incorporation into liposomes or by complexing with cationic lipids.

•Rheumatoid arthritis (RA).

5 Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the synovium: high efficiency of gene transfer and expression for several
10 months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, *J. Clin. Invest.* 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple administrations may be necessary. Retrovirus and adeno-associated virus
15 vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

•Psoriasis

20 The psoriatic plaque is a particularly good candidate for ribozyme or vector delivery. The stratum corneum of the plaque is thinned, providing access to the proliferating keratinocytes. T-cells and dermal dendrocytes can be efficiently targeted by trans-epidermal diffusion .

Organ culture systems for biopsy specimens of psoriatic and normal skin are described in current literature (Nickoloff et al., 1993 *Supra*).
25 Primary human keratinocytes are easily obtained and will be grown into epidermal sheets in tissue culture. In addition to these tissue culture models, the flaky skin mouse develops psoriatic skin in response to UV light. This model would allow demonstration of animal efficacy for ribozyme treatments of psoriasis.

30 •Gene Therapy.

Immune responses limit the efficacy of many gene transfer techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus

vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette that modulates inflammatory and immune responses into existing adenovirus or retrovirus constructs will greatly enhance their potential.

- 5 Thus, ribozymes of the present invention that cleave TNF- α mRNA and thereby TNF- α activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits TNF- α function is described above; available cellular and activity assays
10 are number, reproducible, and accurate. Animal models for TNF- α function and for each of the suggested disease targets exist and can be used to optimize activity.

Example 5: p210bcr-abl

- 15 Chronic myelogenous leukemia exhibits a characteristic disease course, presenting initially as a chronic granulocytic hyperplasia, and invariably evolving into an acute leukemia which is caused by the clonal expansion of a cell with a less differentiated phenotype (*i.e.*, the blast crisis stage of the disease). CML is an unstable disease which ultimately progresses to a terminal stage which resembles acute leukemia. This
20 lethal disease affects approximately 16,000 patients a year. Chemotherapeutic agents such as hydroxyurea or busulfan can reduce the leukemic burden but do not impact the life expectancy of the patient (*e.g.* approximately 4 years). Consequently, CML patients are candidates for bone marrow transplantation (BMT) therapy. However, for those patients
25 which survive BMT, disease recurrence remains a major obstacle (Apperley et al., 1988 *Br. J. Haematol.* 69, 239).

- 30 The Philadelphia (Ph) chromosome which results from the translocation of the *abl* oncogene from chromosome 9 to the *bcr* gene on chromosome 22 is found in greater than 95% of CML patients and in 10-25% of all cases of acute lymphoblastic leukemia [(ALL); Fourth International Workshop on Chromosomes in Leukemia 1982, *Cancer Genet. Cytogenet.* 11, 316]. In virtually all Ph-positive CMLs and approximately 50% of the Ph-positive ALLs, the leukemic cells express *bcr-abl* fusion mRNAs in which exon 2 (b2-a2 junction) or exon 3 (b3-a2 junction) from the major breakpoint cluster region of the *bcr* gene is spliced
35

- to exon 2 of the *abl* gene. Heisterkamp et al., 1985 Nature 315, 758; Shtivelman et al., 1987, Blood 69, 971). In the remaining cases of Ph-positive ALL, the first exon of the *bcr* gene is spliced to exon 2 of the *abl* gene (Hooberman et al., 1989 Proc. Nat. Acad. Sci. USA 86, 4259; 5 Heisterkamp et al., 1988 Nucleic Acids Res. 16, 10069).

- The b3-a2 and b2-a2 fusion mRNAs encode 210 kd *bcr-abl* fusion proteins which exhibit oncogenic activity (Daley et al., 1990 Science 247, 824; Heisterkamp et al., 1990 Nature 344, 251). The importance of the *bcr-abl* fusion protein (*p210^{bcr-abl}*) in the evolution and maintenance of the 10 leukemic phenotype in human disease has been demonstrated using antisense oligonucleotide inhibition of *p210^{bcr-abl}* expression. These inhibitory molecules have been shown to inhibit the in vitro proliferation of leukemic cells in bone marrow from CML patients. Szczylak et al., 1991 Science 253, 562).
- 15 Reddy, U.S. Patent 5,246,921 (hereby incorporated by reference herein) describes use of ribozymes as therapeutic agents for leukemias, such as chronic myelogenous leukemia (CML) by targeting the specific junction region of *bcr-abl* fusion transcripts. It indicates causing cleavage by a ribozyme at or near the breakpoint of such a hybrid chromosome, 20 specifically it includes cleavage at the sequence GUX, where X is A, U or G. The one example presented is to cleave the sequence 5' AGC AG AGUU (cleavage site) CAA AAGCCU-3'.

- Scanlon WO 91/18625, WO 91/18624, and WO 91/18913 and Snyder et al., WO93/03141 and WO94/13793 describe a ribozyme effective 25 to cleave oncogenic variants of H-ras RNA. This ribozyme is said to inhibit H-ras expression in response to external stimuli.

- The invention features use of ribozymes to inhibit the development or expression of a transformed phenotype in man and other animals by modulating expression of a gene that contributes to the expression of CML. 30 Cleavage of targeted mRNAs expressed in pre-neoplastic and transformed cells elicits inhibition of the transformed state.

The invention can be used to treat cancer or pre-neoplastic conditions. Two preferred administration protocols can be used, either in vivo administration to reduce the tumor burden, or ex vivo treatment to

eradicate transformed cells from tissues such as bone marrow prior to reimplantation.

This invention features an enzymatic RNA molecule (or ribozyme) which cleaves mRNA associated with development or maintenance of CML. The mRNA targets are present in the 425 nucleotides surrounding the fusion sites of the *bcr* and *abl* sequences in the b2-a2 and b3-a2 recombinant mRNAs. Other sequences in the 5' portion of the *bcr* mRNA or the 3' portion of the *abl* mRNA may also be targeted for ribozyme cleavage. Cleavage at any of these sites in the fusion mRNA molecules will result in inhibition of translation of the fusion protein in treated cells.

The invention provides a class of chemical cleaving agents which exhibit a high degree of specificity for the mRNA causative of CML. Such enzymatic RNA molecules can be delivered exogenously or endogenously to afflicted cells. In the preferred hammerhead motif the small size (less than 40 nucleotides, preferably between 32 and 36 nucleotides in length) of the molecule allows the cost of treatment to be reduced.

The smallest ribozyme delivered for any type of treatment reported to date (by Rossi et al., 1992 *supra*) is an *in vitro* transcript having a length of 142 nucleotides. Synthesis of ribozymes greater than 100 nucleotides in length is very difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. Delivery of ribozymes by expression vectors is primarily feasible using only *ex vivo* treatments. This limits the utility of this approach. In this invention, an alternative approach uses smaller ribozyme motifs and exogenous delivery. The simple structure of these molecules also increases the ability of the ribozyme to invade targeted regions of the mRNA structure. Thus, unlike the situation when the hammerhead structure is included within longer transcripts, there are no non-ribozyme flanking sequences to interfere with correct folding of the ribozyme structure, as well as complementary binding of the ribozyme to the mRNA target.

The enzymatic RNA molecules of this invention can be used to treat human CML or precancerous conditions. Affected animals can be treated at the time of cancer detection or in a prophylactic manner. This timing of treatment will reduce the number of affected cells and disable cellular

replication. This is possible because the ribozymes are designed to disable those structures required for successful cellular proliferation.

Ribozymes of this invention block to some extent p210^{bcr-abl} expression and can be used to treat disease or diagnose such disease.

- 5 Ribozymes will be delivered to cells in culture and to tissues in animal models of CML. Ribozyme cleavage of *bcr/abl* mRNA in these systems may prevent or alleviate disease symptoms or conditions.

The sequence of human *bcr/abl* mRNA can be screened for accessible sites using a computer folding algorithm. Regions of the mRNA 10 that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Table 29 (All sequences are 5' to 3' in the tables). The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

15 The sequences of the chemically synthesized ribozymes most useful in this study are shown in Table 30. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of 20 hammerhead ribozymes listed in Table 30 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 30 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the 25 ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against *bcr-abl* mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance as described above. These ribozymes cleave *bcr-abl* target sequences *in vitro*.

30 The ribozymes are tested for function *in vivo* by exogenous delivery to cells expressing *bcr-abl*. Ribozymes are delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. Expression of *bcr-abl* is monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. Levels of

bcr-abl mRNA are assessed by Northern analysis, RNase protection, by primer extension analysis or by quantitative RT-PCR techniques. Ribozymes that block the induction of p210^{bcr-abl} protein and mRNA by more than 20% are identified.

5 Example 6: RSV

This invention relates to the use of ribozymes as inhibitors of respiratory syncytial virus (RSV) production, and in particular, the inhibition of RSV replication.

RSV is a member of the virus family paramyxoviridae and is classified
10 under the genus *Pneumovirus* (for a review see McIntosh and Chanock, 1990 in *Virology* ed. B.N. Fields, pp. 1045, Raven Press Ltd. NY). The infectious virus particle is composed of a nucleocapsid enclosed within an envelope. The nucleocapsid is composed of a linear negative single-stranded non-segmented RNA associated with repeating subunits of
15 capsid proteins to form a compact structure and thereby protect the RNA from nuclease degradation. The entire nucleocapsid is enclosed by the envelope. The size of the virus particle ranges from 150 - 300 nm in diameter. The complete life cycle of RSV takes place in the cytoplasm of infected cells and the nucleocapsid never reaches the nuclear
20 compartment (Hall, 1990 in *Principles and Practice of Infectious Diseases* ed. Mandell et al., Churchill Livingstone, NY).

The RSV genome encodes ten viral proteins essential for viral production. RSV protein products include two structural glycoproteins (G and F) found in the envelope spikes, two matrix proteins [M and M2 (22K)]
25 found in the inner membrane, three proteins localized in the nucleocapsid (N, P and L), one protein that is present on the surface of the infected cell (SH), and two nonstructural proteins [NS1 (1C) and NS2 (1B)] found only in the infected cell. The mRNAs for the 10 RSV proteins have similar 5' and 3' ends. UV-inactivation studies suggest that a single promoter is used
30 with multiple transcription initiation sites (Barik et al., 1992 J. Virol. 66, 6813). The order of transcription corresponding to the protein assignment on the genomic RNA is 1C, 1B, N, P, M, SH, G, F, 22K and L genes (Huang et al., 1985 Virus Res. 2, 157) and transcript abundance corresponds to the order of gene assignment (for example the 1C and 1B mRNAs are
35 much more abundant than the L mRNA. Synthesis of viral message begins

immediately after RSV infection of cells and reaches a maximum at 14 hours post-infection (McIntosh and Chanock, *supra*).

- There are two antigenic subgroups of RSV, A and B, which can circulate simultaneously in the community in varying proportions in different years (McIntosh and Chanock, *supra*). Subgroup A usually predominates. Within the two subgroups there are numerous strains. By the limited sequence analysis available it seems that homology at the nucleotide level is more complete within than between subgroups, although sequence divergence has been noted within subgroups as well. Antigenic determinates result primarily from both surface glycoproteins, F and G. For F, at least half of the neutralization epitopes have been stably maintained over a period of 30 years. For G however, A and B subgroups may be related antigenically by as little as a few percent. On the nucleotide level, however, the majority of the divergence in the coding region of G is found in the sequence for the extracellular domain (Johnson et al., 1987, *Proc. Natl. Acad. Sci. USA* 84, 5625).

- Respiratory Syncytial Virus (RSV) is the major cause of lower respiratory tract illness during infancy and childhood (Hall, *supra*) and as such is associated with an estimated 90,000 hospitalizations and 4500 deaths in the United States alone (Update: respiratory syncytial virus activity - United States, 1993, *Mmwr Morb Mortal Wkly Rep*, 42, 971). Infection with RSV generally outranks all other microbial agents leading to both pneumonia and bronchitis. While primarily affecting children under two years of age, immunity is not complete and reinfection of older children and adults, especially hospital care givers (McIntosh and Chanock, *supra*), is not uncommon. Immunocompromised patients are severely affected and RSV infection is a major complication for patients undergoing bone marrow transplantation .

- Uneventful RSV respiratory disease resembles a common cold and recovery is in 7 to 12 days. Initial symptoms (rhinorrhea, nasal congestion, slight fever, etc.) are followed in 1 to 3 days by lower respiratory tract signs of infection that include a cough and wheezing. In severe cases, these mild symptoms quickly progress to tachypnea, cyanosis, and listlessness and hospitalization is required. In infants with underlying cardiac or respiratory disease, the progression of symptoms is especially rapid and can lead to respiratory failure by the second or third day of illness. With

modern intensive care however, overall mortality is usually less than 5% of hospitalized patients (McIntosh and Chanock, *supra*).

At present, neither an efficient vaccine nor a specific antiviral agent is available. An immune response to the viral surface glycoproteins can 5 provide resistance to RSV in a number of experimental animals, and a subunit vaccine has been shown to be effective for up to 6 months in children previously hospitalized with an RSV infection (Tristam *et al.*, 1993, J. Infect. Dis. 167, 191). An attenuated bovine RSV vaccine has also been shown to be effective in calves for a similar length of time (Kubota *et al.*, 10 1992 J. Vet. Med. Sci. 54, 957). Previously however, a formalin-inactivated RSV vaccine was implicated in greater frequency of severe disease in subsequent natural infections with RSV (Connors *et al.*, 1992 J. Virol. 66, 7444).

The current treatment for RSV infection requiring hospitalization is the 15 use of aerosolized ribavirin, a guanosine analog [Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY]. Ribavirin therapy is associated with a decrease in the severity of the symptoms, improved arterial oxygen and a decrease in the amount of viral shedding at the end of the treatment 20 period. It is not certain, however, whether ribavirin therapy actually shortens the patients' hospital stay or diminishes the need for supportive therapies (McIntosh and Chanock, *supra*). The benefits of ribavirin therapy are especially clear for high risk infants, those with the most serious symptoms or for patients with underlying bronchopulmonary or cardiac 25 disease. Inhibition of the viral polymerase complex is supported as the main mechanism for inhibition of RSV by ribavirin, since viral but not cellular polypeptide synthesis is inhibited by ribavirin in RSV-infected cells (Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY]. Since 30 ribavirin is at least partially effective against RSV infection when delivered by aerosolization, it can be assumed that the target cells are at or near the epithelial surface. In this regard, RSV antigen had not spread any deeper than the superficial layers of the respiratory epithelium in autopsy studies of fatal pneumonia (McIntosh and Chanock, *supra*).

35 Jennings *et al.*, WO 94/13688 indicates that targets for specific types of ribozymes include respiratory syncytial virus.

- The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting production of respiratory syncytial virus (RSV). Such ribozymes can be used in a method for treatment of diseases caused by these related viruses in man and other animals. The
- 5 invention also features cleavage of the genomic RNA and mRNA of these viruses by use of ribozymes. In particular, the ribozyme molecules described are targeted to the *NS1 (1C), NS2 (1B) and N* viral genes. These genes are known in the art (for a review see McIntosh and Chanock, 1990 *supra*).
- 10 Ribozymes that cleave the specified sites in RSV mRNAs represent a novel therapeutic approach to respiratory disorders. Applicant indicates that ribozymes are able to inhibit the activity of RSV and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described
- 15 that other ribozymes that cleave these sites in RSV mRNAs encoding 1C, 1B and N proteins may be readily designed and are within the invention. Also, those of ordinary skill in the art, will find that it is clear from the examples described that ribozymes cleaving other mRNAs encoded by RSV (*P, M, SH, G, F, 22K and L*) and the genomic RNA may be readily
- 20 designed and are within the invention.
- In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 31, 33, 35, 37 and 38. Examples of such ribozymes are shown in Tables 32, 34, 36-38. Examples of such ribozymes consist essentially of sequences defined in these
- 25 Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.
- 30 Ribozymes of this invention block to some extent RSV production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of respiratory disorders. Ribozyme cleavage of RSV encoded mRNAs or the genomic RNA in these systems may alleviate disease symptoms.

While all ten RSV encoded proteins (1C, 1B, N, P, M, SH, 22K, F, G, and L) are essential for viral life cycle and are all potential targets for ribozyme cleavage, certain proteins (mRNAs) are more favorable for ribozyme targeting than the others. For example RSV encoded proteins 1C, 1B, SH and 22K are not found in other members of the family paramyxoviridae and appear to be unique to RSV. In contrast the ectodomain of the G protein and the signal sequence of the F protein show significant sequence divergence at the nucleotide level among various RSV sub-groups (Johnson et al., 1987 *supra*). RSV proteins 1C, 1B and N are highly conserved among various subtypes at both the nucleotide and amino acid levels. Also, 1C, 1B and N are the most abundant of all RSV proteins.

The sequence of human RSV mRNAs encoding 1C, 1B and N proteins are screened for accessible sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 31, 33, 34, 37 and 38 (All sequences are 5' to 3' in the tables.) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman et al., 1987 J. Am. Chem. Soc., 109, 7845-7854 and in Scaringe et al., 1990 Nucleic Acids Res., 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G5 and a U for A14 (numbering from Hertel et al., 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowriira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). Hairpin ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant

groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-o-methyl, 2'-H (for a review see Usman and Cedergren, 1992 *TIBS* 17, 34). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography and are resuspended in water.

- 5 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 32, 34, 36, 37 and 38. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of
10 hammerhead ribozymes listed in Tables 32 and 34 (5'-GGCCGAAAGGCC-
 3') can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 37 and 38 (5'-CACGUUGUG-3') can be altered (substitution,
15 deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequences listed in Tables 32, 34, 36, 37 and 38 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.
20 By engineering ribozyme motifs we have designed several ribozymes directed against RSV encoded mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave target sequences *in vitro* is evaluated.

Numerous common cell lines can be infected with RSV for experimental purposes. These include *HeLa*, *Vero* and several primary epithelial cell lines. A cotton rat animal model of experimental human RSV infection is also available, and the bovine RSV is quite homologous to the human viruses. Rapid clinical diagnosis is through the use of kits designed for the immunofluorescence staining of RSV-infected cells or an ELISA assay, both of which are adaptable for experimental study. RSV encoded mRNA levels will be assessed by Northern analysis, RNAse protection, primer extension analysis or quantitative RT-PCR. Ribozymes that block the induction of RSV activity and/or 1C, 1B and N protein encoding mRNAs by more than 90% will be identified.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Draper et al., PCT WO93/23569. The details will not be repeated here, but include altering the length of the ribozyme binding arms or chemically synthesizing 5 ribozymes with modifications that prevent their degradation by serum ribonucleases (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 *Nature* 344, 565; Pieken et al., 1991 *Science* 253, 314; Usman and Cedergren, 1992 *Trends in Biochem. Sci.* 17, 334; Usman et al., International Publication No. WO 93/15187; and 10 Rossi et al., International Publication No. WO 91/03162, as well as Jennings et al., WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.), modifications which enhance their efficacy in cells, and removal of 15 stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan, et al., PCT WO94/02595, incorporated by reference herein, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to 20 those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. The RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or stent. 25 Alternative routes of delivery include, but are not limited to, intravenous injection, intramuscular injection, subcutaneous injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan, et al., *supra* and Draper, et al., 30 *supra* which have been incorporated by reference herein.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II 35 (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given

pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells

5 (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. U S A, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet

10 et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. U S A, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. U S A, 90, 6340-4; L'Huillier et al., 1992 EMBO J., 11, 4411-8; Lisziewicz et al., 1993 Proc. Natl. Acad. Sci. U. S. A., 90, 8000-4). The above ribozyme transcription units can be

15 incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral, or alpha virus vectors).

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves target RNA is inserted into a plasmid DNA vector, a retrovirus DNA viral vector, an adenovirus DNA viral vector or an adeno-associated virus vector or alpha virus vector. These and other vectors have been used to transfer genes to live animals (for a review see Friedman, 1989 Science 244, 1275-1281; Roemer and Friedman, 1992 Eur. J. Biochem. 208, 211-225) and leads to transient or stable gene expression. The vectors are delivered as recombinant viral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant virus particles are locally administered to the site of treatment, e.g., through the

20 use of a catheter, stent or infusion pump.

25

30

Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By

35

using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role
5 (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled
10 with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNA associated with ICAM-1, relA, TNF- α , p210, bcr-abl or RSV related condition. Such RNA is detected
15 by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second
20 ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNase protection assay so that full-length and
25 cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype
30 (i.e., ICAM-1, rel A, TNF α , p210bcr-abl or RSV) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will
35

decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

II. Chemical Synthesis Of Ribozymes

- 5 There follows the chemical synthesis, deprotection, and purification of RNA, enzymatic RNA or modified RNA molecules in greater than milligram quantities with high biological activity. Applicant has determined that the synthesis of enzymatically active RNA in high yield and quantity is dependent upon certain critical steps used during its preparation.
- 10 Specifically, it is important that the RNA phosphoramidites are coupled efficiently in terms of both yield and time, that correct exocyclic amino protecting groups be used, that the appropriate conditions for the removal of the exocyclic amino protecting groups and the alkylsilyl protecting groups on the 2'-hydroxyl are used, and that the correct work-up and
- 15 purification procedure of the resulting ribozyme be used.

To obtain a correct synthesis in terms of yield and biological activity of a large RNA molecule (*i.e.*, about 30 to 40 nucleotide bases), the protection of the amino functions of the bases requires either amide or substituted amide protecting groups, which must be, on the one hand, stable enough to survive the conditions of synthesis, and on the other hand, removable at the end of the synthesis. These requirements are met by the amide protecting groups shown in Figure 8, in particular, benzoyl for adenosine, isobutyryl or benzoyl for cytidine, and isobutyryl for guanosine, which may be removed at the end of the synthesis by incubating the RNA in NH₃/EtOH (ethanolic ammonia) for 20 h at 65 °C. In the case of the phenoxyacetyl type protecting groups shown in Figure 8 on guanosine and adenosine and acetyl protecting groups on cytidine, an incubation in ethanolic ammonia for 4 h at 65 °C is used to obtain complete removal of these protecting groups. Removal of the alkylsilyl 2'-hydroxyl protecting groups can be accomplished using a tetrahydrofuran solution of TBAF at room temperature for 8-24 h.

The most quantitative procedure for recovering the fully deprotected RNA molecule is by either ethanol precipitation, or an anion exchange cartridge desalting, as described in Scaringe *et al.* *Nucleic Acids Res.* 1990, 18, 5433-5341. The purification of the long RNA sequences may be

accomplished by a two-step chromatographic procedure in which the molecule is first purified on a reverse phase column with either the trityl group at the 5' position on or off. This purification is accomplished using an acetonitrile gradient with triethylammonium or bicarbonate salts as the
5 aqueous phase. In the case of the trityl on purification, the trityl group may be removed by the addition of an acid and drying of the partially purified RNA molecule. The final purification is carried out on an anion exchange column, using alkali metal perchlorate salt gradients to elute the fully purified RNA molecule as the appropriate metal salts, e.g. Na⁺, Li⁺ etc. A
10 final de-salting step on a small reverse-phase cartridge completes the purification procedure. Applicant has found that such a procedure not only fails to adversely affect activity of a ribozyme, but may improve its activity to cleave target RNA molecules.

Applicant has also determined that significant (see Tables 39-41)
15 improvements in the yield of desired full length product (FLP) can be obtained by:

1. Using 5-S-alkyltetrazole at a delivered or effective concentration of 0.25-0.5 M or 0.15-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. (By delivered is meant that the
20 actual amount of chemical in the reaction mix is known. This is possible for large scale synthesis since the reaction vessel is of size sufficient to allow such manipulations. The term effective means that available amount of chemical actually provided to the reaction mixture that is able to react with the other reagents present in the mixture. Those skilled in the art will
25 recognize the meaning of these terms from the examples provided herein.) The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m. Alkyl, as used herein, refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1
30 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including
35 straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to

- 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an
- 5 unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the
- 10 substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated π electron

15 system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as

20 described above. Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur,

25 and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

30 2. Using 5-S-alkyltetrazole at an effective, or final, concentration of 0.1-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m.

35 3. Using alkylamine (MA, where alkyl is preferably methyl, ethyl, propyl or butyl) or NH₄OH/alkylamine (AMA, with the same preferred alkyl groups as noted for MA) @ 65 °C for 10-15 m to remove the exocyclic

amino protecting groups (vs 4-20 h @ 55-65 °C using NH₄OH/EtOH or NH₃/EtOH, *vide supra*). Other alkylamines, e.g. ethylamine, propylamine, butylamine etc. may also be used.

4. Using anhydrous triethylamine•hydrogen fluoride (aHF•TEA)
- 5 @ 65 °C for 0.5-1.5 h to remove the 2'-hydroxyl alkylsilyl protecting group (vs 8 - 24 h using TBAF, *vide supra* or TEA•3HF for 24 h (Gasparutto et al. *Nucleic Acids Res.* 1992, 20, 5159-5166). Other alkylamine•HF complexes may also be used, e.g. trimethylamine or diisopropylethylamine.

5. The use of anion-exchange resins to purify and/or analyze the
10 fully deprotected RNA. These resins include, but are not limited to, quartenary or tertiary amino derivatized stationary phases such as silica or polystyrene. Specific examples include Dionex-NA100®, Mono-Q®, Poros-Q®.

- Thus, the invention features an improved method for the coupling of
15 RNA phosphoramidites; for the removal of amide or substituted amide protecting groups; and for the removal of 2'-hydroxyl alkylsilyl protecting groups. Such methods enhance the production of RNA or analogs of the type described above (e.g., with substituted 2'-groups), and allow efficient synthesis of large amounts of such RNA. Such RNA may also have
20 enzymatic activity and be purified without loss of that activity. While specific examples are given herein, those in the art will recognize that equivalent chemical reactions can be performed with the alternative chemicals noted above, which can be optimized and selected by routine experimentation.

- In another aspect, the invention features an improved method for the purification or analysis of RNA or enzymatic RNA molecules (e.g. 28-70 nucleotides in length) by passing said RNA or enzymatic RNA molecule over an HPLC, e.g., reverse phase and/or an anion exchange chromatography column. The method of purification improves the catalytic activity of enzymatic RNAs over the gel purification method (see Figure 10).

- 30 Draper et al., PCT WO93/23569, incorporated by reference herein, disclosed reverse phase HPLC purification. The purification of long RNA molecules may be accomplished using anion exchange chromatography, particularly in conjunction with alkali perchlorate salts. This system may be used to purify very long RNA molecules. In particular, it is advantageous to

use a Dionex NucleoPak 100[©] or a Pharmacia Mono Q[®] anion exchange column for the purification of RNA by the anion exchange method. This anion exchange purification may be used following a reverse-phase purification or prior to reverse phase purification. This method results in the 5 formation of a sodium salt of the ribozyme during the chromatography. Replacement of the sodium alkali earth salt by other metal salts, e.g., lithium, magnesium or calcium perchlorate, yields the corresponding salt of the RNA molecule during the purification.

In the case of the 2-step purification procedure, in which the first step 10 is a reverse phase purification followed by an anion exchange step, the reverse phase purification is best accomplished using polymeric, e.g. polystyrene based, reverse-phase media, using either a 5'-trityl-on or 5'-trityl-off method. Either molecule may be recovered using this reverse-phase method, and then, once detritylated, the two fractions may be pooled 15 and then submitted to an anion exchange purification step as described above.

The method includes passing the enzymatically active RNA molecule over a reverse phase HPLC column; the enzymatically active RNA molecule is produced in a synthetic chemical method and not by an 20 enzymatic process; and the enzymatic RNA molecule is partially blocked, and the partially blocked enzymatically active RNA molecule is passed over a reverse phase HPLC column to separate it from other RNA molecules.

In more preferred embodiments, the enzymatically active RNA 25 molecule, after passage over the reverse phase HPLC column, is deprotected and passed over a second reverse phase HPLC column (which may be the same as the reverse phase HPLC column), to remove the enzymatic RNA molecule from other components. In addition, the column is a silica or organic polymer-based C4, C8 or C18 column having 30 a porosity of at least 125 Å, preferably 300 Å, and a particle size of at least 2 µm, preferably 5 µm.

Activation

The synthesis of RNA molecules may be accomplished chemically or 35 enzymatically. In the case of chemical synthesis the use of tetrazole as an activator of RNA phosphoramidites is known (Usman *et al.* *J. Am. Chem.*

Soc. 1987, 109, 7845-7854). In this, and subsequent reports, a 0.5 M solution of tetrazole is allowed to react with the RNA phosphoramidite and couple with the polymer bound 5'-hydroxyl group for 10 m. Applicant has determined that using 0.25-0.5 M solutions of 5-S-alkyltetrazoles for only 5 min gives equivalent or better results. The following exemplifies the procedure.

Example 7: Synthesis of RNA and Ribozymes Using 5-S-Alkyltetrazoles as Activating Agent

The method of synthesis used follows the general procedure for RNA synthesis as described in Usman et al., 1987*supra* and in Scaringe et al., *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The major difference used was the activating agent, 5-S-ethyl or -methyltetrazole @ 0.25 M concentration for 5 min.

All small scale syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 μ mol scale protocol with a reduced 5 min coupling step for alkylsilyl protected RNA and 2.5 m coupling step for 2'-O-methylated RNA. A 6.5-fold excess (162.5 μ L of 0.1 M = 32.5 μ mol) of phosphoramidite and a 40-fold excess of S-ethyl tetrazole (400 μ L of 0.25 M = 100 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% N-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems.

All large scale syntheses were conducted on a modified (eight amidite port capacity) 390Z (ABI) synthesizer using a 25 μ mol scale protocol with a 5-15 min coupling step for alkylsilyl protected RNA and 7.5 m coupling step for 2'-O-methylated RNA. A six-fold excess (1.5 mL of 0.1 M = 150 μ mol) of phosphoramidite and a forty-five-fold excess of S-ethyl tetrazole (4.5 mL of

0.25 M = 1125 μmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 390Z, determined by colorimetric quantitation of the trityl fractions, was 95.0-96.7%. Oligonucleotide synthesis reagents for the 390Z: Detritylation solution was 5 2% DCA in methylene chloride; capping was performed with 16% *N*-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. *S*-Ethyl tetrazole solution (0.25-0.5 M in acetonitrile) was made up 10 from the solid obtained from Applied Biosystems.

Deprotection

The first step of the deprotection of RNA molecules may be accomplished by removal of the exocyclic amino protecting groups with either NH₄OH/EtOH:3/1 (Usman *et al.* *J. Am. Chem. Soc.* 1987, 109, 7845-15 7854) or NH₃/EtOH (Scaringe *et al.* *Nucleic Acids Res.* 1990, 18, 5433-5341) for ~20 h @ 55-65 °C. Applicant has determined that the use of methylamine or NH₄OH/methylamine for 10-15 min @ 55-65 °C gives equivalent or better results. The following exemplifies the procedure.

Example 8: RNA and Ribozyme Deprotection of Exocyclic Amino Protecting Groups Using Methylamine (MA) or NH₄OH/Methylamine (AMA)

The polymer-bound oligonucleotide, either trityl-on or off, was suspended in a solution of methylamine (MA) or NH₄OH/methylamine (AMA) @ 55-65 °C for 5-15 min to remove the exocyclic amino protecting groups. The polymer-bound oligoribonucleotide was transferred from the 25 synthesis column to a 4 mL glass screw top vial. NH₄OH and aqueous methylamine were pre-mixed in equal volumes. 4 mL of the resulting reagent was added to the vial, equilibrated for 5 m at RT and then heated at 55 or 65 °C for 5-15 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed with 1.0 mL 30 of EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder. The same procedure was followed for the aqueous methylamine reagent.

Table 40 is a summary of the results obtained using the improvements 35 outlined in this application for base deprotection.

The second step of the deprotection of RNA molecules may be accomplished by removal of the 2'-hydroxyl alkylsilyl protecting group using TBAF for 8-24 h (Usman *et al.* *J. Am. Chem. Soc.* 1987, 109, 7845-7854). Applicant has determined that the use of anhydrous TEA•HF in *N*-methylpyrrolidine (NMP) for 0.5-1.5 h @ 55-65 °C gives equivalent or better results. The following exemplifies this procedure.

Example 9: RNA and Ribozyme Deprotection of 2'-Hydroxyl Alkylsilyl Protecting Groups Using Anhydrous TEA•HF

To remove the alkylsilyl protecting groups, the ammonia-deprotected oligoribonucleotide was resuspended in 250 µL of 1.4 M anhydrous HF solution (1.5 mL *N*-methylpyrrolidine, 750 µL TEA and 1.0 mL TEA•3HF) and heated to 65 °C for 1.5 h. 9 mL of 50 mM TEAB was added to quench the reaction. The resulting solution was loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) prewashed with 10 mL of 50 mM TEAB. After washing the cartridge with 10 mL of 50 mM TEAB, the RNA was eluted with 10 mL of 2 M TEAB and dried down to a white powder.

Table 41 is a summary of the results obtained using the improvements outlined in this application for alkylsilyl deprotection.

Example 10: HPLC Purification, Anion Exchange column

For a small scale synthesis, the crude material was diluted to 5 mL with diethylpyrocarbonate treated water. The sample was injected onto either a Pharmacia Mono Q® 16/10 or Dionex NucleoPac® column with 100% buffer A (10 mM NaClO₄). A gradient from 180-210 mM NaClO₄ at a rate of 0.85 mM/void volume for a Pharmacia Mono Q® anion-exchange column or 100-150 mM NaClO₄ at a rate of 1.7 mM/void volume for a Dionex NucleoPac® anion-exchange column was used to elute the RNA. Fractions were analyzed by a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing full length product at ≥80% by peak area were pooled.

For a trityl-off large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose® Fast Flow column. The column was thoroughly washed with 10 mM sodium perchlorate buffer. The oligonucleotide was eluted from the column with

300 mM sodium perchlorate. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material in the synthesis. The eluent was diluted four fold in sterile H₂O to lower the salt concentration and applied to a Pharmacia Mono Q® 16/10 column. A 5 gradient from 10-185 mM sodium perchlorate was run over 4 column volumes to elute shorter sequences, the full length product was then eluted in a gradient from 185-214 mM sodium perchlorate in 30 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing over 85% full length material 10 were pooled. The pool was applied to a Pharmacia RPC® column for desalting.

For a trityl-on large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose® Fast Flow 15 column. The column was thoroughly washed with 20 mM NH₄CO₃H/10% CH₃CN buffer. The oligonucleotide was eluted from the column with 1.5 M NH₄CO₃H/10% acetonitrile. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material present in the synthesis. The oligonucleotide was then applied to a Pharmacia Resource 20 RPC column. A gradient from 20-55% B (20 mM NH₄CO₃H/25% CH₃CN, buffer A = 20 mM NH₄CO₃H/10% CH₃CN) was run over 35 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing over 60% full length 25 material were pooled. The pooled fractions were then submitted to manual detritylation with 80% acetic acid, dried down immediately, resuspended in sterile H₂O, dried down and resuspended in H₂O again. This material was analyzed on a HP 1090-HPLC with a Dionex NucleoPac® column. The material was purified by anion exchange chromatography as in the trityl-off scheme (*vide supra*).

30 Example 11 Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 μM, 200 nM, 40 nM or 8 nM and the final substrate RNA 35 concentrations were ~ 1 nM. Total reaction volumes were 50 μL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were

initiated by mixing substrate and ribozyme solutions at t = 0. Aliquots of 5 µL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each aliquot was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis. Quantitative analyses were
5 performed using a phosphorimager (Molecular Dynamics).

Example 12: One pot deprotection of RNA

Applicant has shown that aqueous methyl amine is an efficient reagent to deprotect bases in an RNA molecule. However, in a time consuming step (2-24 hrs), the RNA sample needs to be dried completely
10 prior to the deprotection of the sugar 2'-hydroxyl groups. Additionally, deprotection of RNA synthesized on a large scale (e.g., 100 µmol) becomes challenging since the volume of solid support used is quite large. In an attempt to minimize the time required for deprotection and to simplify
15 the process of deprotection of RNA synthesized on a large scale, applicant describes a one pot deprotection protocol (Fig. 12). According to this protocol, anhydrous methylamine is used in place of aqueous methyl amine. Base deprotection is carried out at 65 °C for 15 min and the reaction is allowed to cool for 10 min. Deprotection of 2'-hydroxyl groups is then carried out in the same container for 90 min in a TEA•3HF reagent.
20 The reaction is quenched with 16 mM TEAB solution.

Referring to Fig. 13, hammerhead ribozyme targeted to site B is synthesized using RNA phosphoramidite chemistry and deprotected using either a two pot or a one pot protocol. Profiles of these ribozymes on an HPLC column are compared. The figure shows that RNAs deprotected by
25 either the one pot or the two pot protocols yield similar full-length product profiles. Applicant has shown that using a one pot deprotection protocol, time required for RNA deprotection can be reduced considerably without compromising the quality or the yield of full length RNA.

Referring to Fig. 14, hammerhead ribozymes targeted to site B (from
30 Fig. 13) are tested for their ability to cleave RNA. As shown in the figure 14, ribozymes that are deprotected using one pot protocol have catalytic activity comparable to ribozymes that are deprotected using a two pot protocol.

Example 12a: Improved protocol for the synthesis of phosphorothioate containing RNA and ribozymes using 5-S-Alkyltetrazoles as Activating Agent

- The two sulfurizing reagents that have been used to synthesize 5 ribophosphorothioates are tetraethylthiuram disulfide (TETD; Vu and Hirschbein, 1991 *Tetrahedron Letter* 31, 3005), and 3H-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage reagent; Vu and Hirschbein, 1991 *supra*). TETD requires long sulfurization times (600 seconds for DNA and 3600 seconds for RNA). It has recently been shown that for sulfurization of DNA 10 oligonucleotides, Beaucage reagent is more efficient than TETD (Wyrzykiewicz and Ravikumar, 1994 *Bioorganic Med. Chem.* 4, 1519). Beaucage reagent has also been used to synthesize phosphorothioate oligonucleotides containing 2'-deoxy-2'-fluoro modifications wherein the wait time is 10 min (Kawasaki et al., 1992 *J. Med. Chem.*).
- 15 The method of synthesis used follows the procedure for RNA synthesis as described herein and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The sulfurization step for RNA described in the literature is a 8 second delivery and 10 min wait steps (Beaucage and Iyer, 1991 *Tetrahedron* 49, 6123). These conditions produced about 20 95% sulfurization as measured by HPLC analysis (Morvan et al., 1990 *Tetrahedron Letter* 31, 7149). This 5% contaminating oxidation could arise from the presence of oxygen dissolved in solvents and/or slow release of traces of iodine adsorbed on the inner surface of delivery lines during 25 previous synthesis.

- A major improvement is the use of an activating agent, 5-S-ethyltetrazole or 5-S-methyltetrazole at a concentration of 0.25 M for 5 min. Additionally, for those linkages which are phosphorothioate, the iodine solution is replaced with a 0.05 M solution of 3H-1,2-benzodithiole-3-one 30 1,1-dioxide (Beaucage reagent) in acetonitrile. The delivery time for the sulfurization step is reduced to 5 seconds and the wait time is reduced to 300 seconds.

- RNA synthesis is conducted on a 394 (ABI) synthesizer using a modified 2.5 μ mol scale protocol with a reduced 5 min coupling step for 35 alkylsilyl protected RNA and 2.5 min coupling step for 2'-O-methylated RNA. A 6.5-fold excess (162.5 μ L of 0.1 M = 32.5 μ mol) of phosphoramidite

and a 40-fold excess of S-ethyl tetrazole (400 μ L of 0.25 M = 100 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394 synthesizer, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394 synthesizer: detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% N-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems. Sulfurizing reagent was obtained from Glen Research.

Average sulfurization efficiency (ASE) is determined using the formula: ASE = (PS/Total)^{1/n-1}

where, PS = integrated ³¹P NMR values of the P=S diester

Total = integration value of all peaks

n = length of oligo

Referring to tables 42 and 43, effects of varying the delivery and the wait time for sulfurization with Beaucage's reagent is described. These data suggest that 5 second wait time and 300 second delivery time is the condition under which ASE is maximum.

Using the above conditions a 36 mer hammerhead ribozyme is synthesized which is targeted to site C. The ribozyme is synthesized to contain phosphorothioate linkages at four positions towards the 5' end. RNA cleavage activity of this ribozyme is shown in Fig. 16. Activity of the phosphorothioate ribozyme is comparable to the activity of a ribozyme lacking any phosphorothioate linkages.

Example 13: Protocol for the synthesis of 2'-N-phtalimido-nucleoside phosphoramidite

The 2'-amino group of a 2'-deoxy-2'-amino nucleoside is normally protected with N-(9-fluorenylmethoxycarbonyl) (Fmoc; Imazawa and Eckstein, 1979 *supra*; Pieken et al., 1991 *Science* 253, 314). This protecting group is not stable in CH₃CN solution or even in dry form during

prolonged storage at -20 °C. These problems need to be overcome in order to achieve large scale synthesis of RNA.

Applicant describes the use of alternative protecting groups for the 2'-amino group of 2'-deoxy-2'-amino nucleoside. Referring to Figure 17, 5 phosphoramidite 17 was synthesized starting from 2'-deoxy-2'-aminonucleoside (12) using transient protection with Markevich reagent (Markiewicz *J. Chem. Res.* 1979, S, 24). An intermediate 13 was obtained in 50% yield, however subsequent introduction of N-phtaloyl (Pht) group by Nefken's method (Nefkens, 1960 *Nature* 185, 306), desilylation (15), 10 dimethoxytrytilation (16) and phosphorylation led to phosphoramidite 17. Since overall yield of this multi-step procedure was low (20%) applicant investigated some alternative approaches, concentrating on selective introduction of N-phtaloyl group without acylation of 5' and 3' hydroxyls.

When 2'-deoxy-2'-amino-nucleoside was reacted with 1.05 15 equivalents of Nefkens reagent in DMF overnight with subsequent treatment with Et₃N (1 hour) only 10-15% of N and 5'(3')-bis-phtaloyl derivatives were formed with the major component being N-Pht-derivative 15. The N,O-bis by-products could be selectively and quantitatively converted to N-Pht derivative 15 by treatment of crude reaction mixture 20 with cat. KCN/MeOH.

A convenient "one-pot" procedure for the synthesis of key intermediate 16 involves selective N-phthaloylation with subsequent dimethoxytrytilation by DMTCI/Et₃N and resulting in the preparation of DMT derivative 16 in 85% overall yield as follows. Standard phosphorylation of 25 16 produced phosphoramidite 17 in 87% yield. One gram of 2'-amino nucleoside, for example 2'-amino uridine (US Biochemicals® part # 77140) was co-evaporated twice from dry dimethyl formamide (Dmf) and dried in vacuo overnight. 50 mls of Aldrich sure-seal Dmf was added to the dry 2'-amino uridine via syringe and the mixture was stirred for 10 minutes 30 to produce a clear solution. 1.0 grams (1:05 eq.) of N-carbethoxyphthalimide (Nefken's reagent, 98% Jannsen Chimica) was added and the solution was stirred overnight. Thin layer chromatography (TLC) showed 90% conversion to a faster moving products (10% ETOH in CHCl₃) and 57 µl of TEA (0.1 eq.) was added to effect closure of the 35 phthalimide ring. After 1 hour an additional 855 µl (1.5 eq.) of TEA was added followed by the addition of 1.53 grams (1.1 eq.) of DMT-Cl

- (Lancaster Synthesis®, 98%). The reaction mixture was left to stir overnight and quenched with ETOH after TLC showed greater than 90% desired product. Dmf was removed under vacuum and the mixture was washed with sodium bicarbonate solution (5% aq., 500 mls) and extracted
- 5 with ethyl acetate (2x 200 mls). A 25mm x 300mm flash column (75 grams Merck flash silica) was used for purification. Compound eluted at 80 to 85% ethyl acetate in hexanes (yield: 80% purity: >95% by ^1H NMR). Phosphoramidites were then prepared using standard protocols described above.
- 10 With phosphoramidite 17 in hand applicant synthesized several ribozymes with 2'-deoxy-2'-amino modifications. Analysis of the synthesis demonstrated coupling efficiency in 97-98% range. RNA cleavage activity of ribozymes containing 2'-deoxy-2'-amino-U modifications at U4 and/or U7 positions (see Figure 1), wherein the 2'-amino positions were either
- 15 protected with Fmoc or Pht, was identical. Additionally, complete deprotection of 2'-deoxy-2'-amino-Uridine was confirmed by base-composition analysis. The coupling efficiency of phosphoramidite 17 was not effected over prolonged storage (1-2 months) at low temperatures.
- Protecting 2' Position with a SEM Group
- 20 There follows a method using the 2'-(trimethylsilyl)ethoxymethyl protecting group (SEM) in the synthesis of oligoribonucleotides, and in particular those enzymatic molecules described above. For the synthesis of RNA it is important that the 2'-hydroxyl protecting group be stable throughout the various steps of the synthesis and base deprotection. At the
- 25 same time, this group should also be readily removed when desired. To that end the *t*-butyldimethylsilyl group has been efficacious (Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 1990, 18, 5433-5441). However, long exposure times to tetra-*n*-butylammonium fluoride (TBAF) are generally required to fully remove this
- 30 protecting group from the 2'-hydroxyl. In addition, the bulky alkyl substituents can prove to be a hindrance to coupling thereby necessitating longer coupling times. Finally, it has been shown that the TBDMS group is base labile and is partially deprotected during treatment with ethanolic ammonia (Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 1990,

18, 5433-5441 and Stawinski,J.; Stromberg,R.; Thelin,M.; Westman,E. *Nucleic Acids Res.* 1988, 16, 9285-9298).

The (trimethylsilyl)ethoxymethyl ether (SEM) seems a suitable substitute. This protecting group is stable to base and all but the harshest acidic conditions. Therefore it is stable under the conditions required for oligonucleotide synthesis. It can be readily introduced and the oxygen carbon bond makes it unable to migrate. Finally, the SEM group can be removed with $\text{BF}_3\text{-OEt}_2$ very quickly.

There follows a method for synthesis of RNA by protecting the 2'-position of a nucleotide during RNA synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group. The method can involve use of standard RNA synthesis conditions as discussed below, or any other equivalent steps. Those in the art are familiar with such steps. The nucleotide used can be any normal nucleotide or may be substituted in various positions by methods well known in the art, e.g., as described by Eckstein *et al.*, International Publication No. WO 92/07065, Perrault *et al.*, *Nature* 1990, 344, 565-568, Pieken *et al.*, *Science* 1991, 253, 314-317, Usman,N.; Cedergren,R.J. *Trends in Biochem. Sci.* 1992, 17, 334-339, Usman *et al.*, PCT WO93/15187, and Sproat,B. *European Patent Application* 92110298.4 .

This invention also features a method for covalently linking a SEM group to the 2'-position of a nucleotide. The method involves contacting a nucleoside with an SEM-containing molecule under SEM bonding conditions. In a preferred embodiment, the conditions are dibutyltin oxide, tetrabutylammonium fluoride and SEM-Cl. Those in the art, however, will recognize that other equivalent conditions can also be used.

In another aspect, the invention features a method for removal of an SEM group from a nucleoside molecule or an oligonucleotide. The method involves contacting the molecule or oligonucleotide with boron trifluoride etherate ($\text{BF}_3\text{-OEt}_2$) under SEM removing conditions, e.g., in acetonitrile.

Referring to Figure 18, there is shown the method for solid phase synthesis of RNA. A 2',5'-protected nucleotide is contacted with a solid phase bound nucleotide under RNA synthesis conditions to form a dinucleotide. The protecting group (R) at the 2'-position in prior art

methods can be a silyl ether, as shown in the Figure. In the method of the present invention, an SEM group is used in place of the silyl ether. Otherwise RNA synthesis can be performed by standard methodology.

Referring to Figure 19, there is shown the synthesis of 2'-O-SEM protected nucleosides and phosphoramidites. Briefly, a 5'-protected nucleoside (1) is protected at the 2'- or 3'-position by contacting with a derivative of SEM under appropriate conditions. Specifically, those conditions include contacting the nucleoside with dibutyltin oxide and SEM chloride. The 2 regioisomers are separated by chromatography and the 2'-protected moiety is converted into a phosphoramidite by standard procedure. The 3'-protected nucleoside is converted into a succinate derivative suitable for derivatization of a solid support.

Referring to Figure 20, a prior art method for deprotection of RNA using silyl ethers is shown. This contrasts with the method shown in Figure 21 in which deprotection of RNA containing an SEM group is performed. In step 1, the base protecting groups and cyanoethyl groups are removed by standard procedure. The SEM group is then removed as shown in the Figure. The details of the synthesis of phosphoramidites and SEM protected nucleosides and their use in synthesis of oligonucleotides and subsequent deprotection of

Example 14: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5'-O-Dimethoxytrityl Uridine (2)

Referring to Figure 19, 5'-O-dimethoxytrityl uridine 1 (1.0 g, 1.83 mmol) in CH₃CN (18 mL) was added dibutyltin oxide (1.0 g, 4.03 mmol) and TBAF (1 M, 2.38 mL, 2.38 mmol). The mixture was stirred for 2 h at RT (about 20-25°C) at which time (trimethylsilyl)ethoxymethyl chloride (SEM-Cl) (487 µL, 2.75 mmol) was added. The reaction mixture was stirred overnight and then filtered and evaporated. Flash chromatography (30% hexanes in ethyl acetate) yielded 347 mg (28.0%) of 2'-hydroxyl protected nucleoside 2 and 314 mg (25.3%) of 3'-hydroxyl protected nucleoside 3.

Example 15: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl) Uridine (4)

Nucleoside 2 was detritylated following standard methods, as shown in Figure 19.

Example 16: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5',3'-O-Acetyl Uridine (5)

Nucleoside 4 was acetylated following standard methods, as shown in Figure 19.

5 Example 17: Synthesis of 5',3'-O-Acetyl Uridine (6)

Referring to Figure 19, the fully protected uridine 5 (32 mg, 0.07 mmol) was dissolved in CH₃CN (700 µL) and BF₃•OEt₂ (17.5 µL, 0.14 mmol) was added. The reaction was stirred 15 m and MeOH was added to quench the reaction. Flash chromatography (5% MeOH in CH₂Cl₂) gave
10 20 mg (88%) of SEM deprotected nucleoside 6.

Example 18: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-3'-O-Succinyl-5'-O-Dimethoxytrityl Uridine (2)

Nucleoside 3 was succinylated and coupled to the support following standard procedures, as shown in Figure 19.

15 Example 19: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5'-O-Dimethoxytrityl Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (8)

Nucleoside 3 was phosphitylated following standard methods, as shown in Figure 19.

20 Example 20: Synthesis of RNA Using 2'-O-SEM Protection

Referring to Figure 18, the method of synthesis used follows the general procedure for RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 1990, 18, 5433-5441. The phosphoramidite 8 was coupled following standard RNA methods to provide a 10-mer of uridylic acid. Syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 µmol scale protocol with a 10 m coupling step. A thirteen-fold excess (325 µL of 0.1 M = 32.5 µmol) of phosphoramidite and a 80-fold excess of tetrazole (400 µL of 0.5 M = 200 µmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, were 98-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% *N*-

Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle.

- 5 Referring to Figure 21, the homopolymer was base deprotected with NH₃/EtOH at 65 °C. The solution was decanted and the support was washed twice with a solution of 1:1:1 H₂O:CH₃CN:MeOH. The combined solutions were dried down and then diluted with CH₃CN (1 mL). BF₃•OEt₂ (2.5 µL, 30 µmol) was added to the solution and aliquots were removed at
10 ten time points. The results indicate that after 30 min deprotection is complete, as shown in Figure 22.

III. Vectors Expressing Ribozymes

There follows a method for expression of a ribozyme in a bacterial or eucaryotic cell, and for production of large amounts of such a ribozyme. In
15 general, the invention features a method for preparing multi-copy cassettes encoding a defined ribozyme structure for production of a ribozyme at a decreased cost. A vector is produced which encodes a plurality of ribozymes which are cleaved at their 3' and 5' ends from an RNA transcript produced from the vector by only one other ribozyme. The system is useful
20 for scaling up production of a ribozyme, which may be either modified or unmodified, *in situ* or *in vitro*. Such vector systems can be used to express a desired ribozyme in a specific cell, or can be used in an *in vitro* system to allow productiuon of large amounts of a desired riboqyne, The vectors of this invention allow a higher yield synthesis of a ribozyme in the form of an
25 RNA transcript which is cleaved *in situ* or *in vitro* before or after transcript isolation.

Thus, this invention is distinct from the prior art in that a single ribozyme is used to process the 3' and 5' ends of each therapeutic, trans-acting or desired ribozyme instead of processing only one end, or only one
30 ribozyme. This allows smaller vectors to be derived with multiple trans-acting ribozymes released by only one other ribozyme from the mRNA transcript. Applicant has also provided methods by which the activity of such ribozymes is increased compared to those in the art, by designing ribozyme-encoding vectors and the corresponding transcript such that

folding of the mRNA does not interfere with processing by the releasing ribozyme.

The stability of the ribozyme produced in this method can be enhanced by provision of sequences at the termini of the ribozymes as
5 described by Draper et al., PCT WO 93/23509, hereby incorporated by reference herein.

The method of this invention is advantageous since it provides high yield synthesis of ribozymes by use of low cost transcription-based protocols, compared to existing chemical ribozyme synthesis, and can use
10 isolation techniques currently used to purify chemically synthesized oligonucleotides. Thus, the method allows synthesis of ribozymes in high yield at low cost for analytical, diagnostic, or therapeutic applications.

The method is also useful for synthesis of ribozymes *in vitro* for ribozyme structural studies, enzymatic studies, target RNA accessibility
15 studies, transcription inhibition studies and nuclease protection studies, much is described by Draper et al., PCT WO 93/23509 hereby incorporated by reference herein.

The method can also be used to produce ribozymes *in situ* either to increase the intracellular concentration of a desired therapeutic ribozyme,
20 or to produce a concatameric transcript for subsequent *in vitro* isolation of unit length ribozyme. The desired ribozyme can be used to inhibit gene expression in molecular genetic analyses or in infectious cell systems, and to test the efficacy of a therapeutic molecule or treat afflicted cells.

Thus, in general, the invention features a vector which includes a
25 bacterial, viral or eucaryotic promoter within a plasmid, cosmid, phagemid, virus, viroid, virusoid or phage vector. Other vectors are equally suitable and include double-stranded, or partially double-stranded DNA, formed by an amplification method such as the polymerase chain reaction, or double-stranded, partially double-stranded or single-stranded RNA, formed by site-directed homologous recombination into viral or viroid RNA genomes.
30 Such vectors need not be circular. Transcriptionally linked to the promoter region is a first ribozyme-encoding region, and nucleotide sequences encoding a ribozyme cleavage sequence which is placed on either side of a region encoding a therapeutic or otherwise desired second ribozyme.

Suitable restriction endonuclease sites can be provided to ease construction of this vector in DNA vectors or in requisite DNA vectors of an RNA expression system. The desired second ribozyme may be any desired type of ribozyme, such as a hammerhead, hairpin, hepatitis delta virus (HDV) or other catalytic center, and can include group I and group II introns, as discussed above. The first ribozyme is chosen to cleave the encoded cleavage sequence, and may also be any desired ribozyme, for example, a *Tetrahymena* derived ribozyme, which may, for example, include an imbedded restriction endonuclease site in the center of a self-recognition sequence to aid in vector construction. This endonuclease site is useful for construction of the vector, and subsequent analysis of the vector.

When the promoter of such a vector is activated an RNA transcript is produced which includes the first and second ribozyme sequences. The first ribozyme sequence is able to act, under appropriate conditions, to cause cleavage at the cleavage sites to release the second ribozyme sequences. These second ribozyme sequences can then act at their target RNA sites, or can be isolated for later use or analysis.

Thus, in one aspect the invention features a vector which includes a first nucleic acid sequence (encoding a first ribozyme having intramolecular cleaving activity), and a second nucleic acid sequence (encoding a second ribozyme having intermolecular cleaving enzymatic activity) flanked by nucleic acid sequences encoding RNA which is cleaved by the first ribozyme to release the second ribozyme from the RNA transcript encoded by the vector. The second ribozyme may be flanked by the first ribozyme either on the 5' side or 3' side. If desired, the first ribozyme may be encoded on a separate vector and may have intermolecular cleaving activity.

As discussed above, the first ribozyme can be chosen to be any self-cleaving ribozyme, and the second ribozyme may be chosen to be any desired ribozyme. The flanking sequences are chosen to include sequences recognized by the first ribozyme. When the vector is caused to express RNA from these nucleic acid sequences, that RNA has the ability under appropriate conditions to cleave each of the flanking regions and thereby release one or more copies of the second ribozyme. If desired, several different second ribozymes can be produced by the same vector, or

several different vectors can be placed in the same vessel or cell to produce different ribozymes.

In preferred embodiments, the vector includes a plurality of the nucleic acid sequences encoding the second ribozyme, each flanked by nucleic acid sequences recognized by the first ribozyme. Most preferably, such a plurality includes at least six to nine or even between 60 - 100 nucleic acid sequences. In other preferred embodiments, the vector includes a promoter which regulates expression of the nucleic acid encoding the ribozymes from the vector; and the vector is chosen from a plasmid, cosmid, phagemid, virus, viroid or phage. In a most preferred embodiment, the plurality of nucleic acid sequences are identical and are arranged in sequential order such that each has an identical end nearest to the promoter. If desired, a poly(A) sequence adjacent to the sequence encoding the first or second ribozyme may be provided to increase stability of the RNA produced by the vector; and a restriction endonuclease site adjacent to the nucleic acid encoding the first ribozyme is provided to allow insertion of nucleic acid encoding the second ribozyme during construction of the vector.

In a second aspect, the invention features a method for formation of a ribozyme expression vector by providing a vector including nucleic acid encoding a first ribozyme, as discussed above, and providing a single-stranded DNA encoding a second ribozyme, as discussed above. The single-stranded DNA is then allowed to anneal to form a partial duplex DNA which can be filled in by a treatment with an appropriate enzyme, such as a DNA polymerase in the presence of dNTPs, to form a duplex DNA which can then be ligated to the vector. Large vectors resulting from this method can then be selected to insure that a high copy number of the single-stranded DNA encoding the second ribozyme is incorporated into the vector.

In a further aspect, the invention features a method for production of ribozymes by providing a vector as described above, expressing RNA from that vector, and allowing cleavage by the first ribozyme to release the second ribozyme.

In preferred embodiments, three different ribozyme motifs are used as cis-cleaving ribozymes. The hammerhead, hairpin, and hepatitis delta

virus (HDV) ribozyme motifs consist of small, well-defined sequences that rapidly self-cleave *in vitro* (Symons, 1992 *Annu. Rev. Biochem.* 61, 641). While structural and functional differences exist among the three ribozyme motifs, they self-process efficiently *in vivo*. All three ribozyme motifs self-
5 process to 87-95% completion in the absence of 3' flanking sequences. *In vitro*, the self-processing constructs described in this invention are significantly more active than those reported by Taira et al., 1990 *supra*; and Altschuler et al., 1992 *Gene* 122, 85. The present invention enables
10 the use of cis-cleaving ribozymes to efficiently truncate RNA molecules at specific sites *in vivo* by ensuring lack of secondary structure which prevents processing.

Isolation of Therapeutic Ribozyme

The preferred method of isolating therapeutic ribozyme is by a chromatographic technique. The HPLC purification methods and reverse
15 HPLC purification methods described by Draper et al., PCT WO 93/23509, hereby incorporated by reference herein, can be used. Alternatively, the attachment of complementary oligonucleotides to cellulose or other chromatography columns allows isolation of the therapeutic second ribozyme, for example, by hybridization to the region between the flanking
20 arms and the enzymatic RNA. This hybridization will select against the short flanking sequences without the desired enzymatic RNA, and against the releasing first ribozyme. The hybridization can be accomplished in the presence of a chaotropic agent to prevent nuclease degradation. The oligonucleotides on the matrix can be modified to minimize nuclease
25 activity, for example, by provision of 2'-O-methyl RNA oligonucleotides. Such modifications of the oligonucleotide attached to the column matrix will allow the multiple use of the column with minimal oligo degradation. Many such modifications are known in the art, but a chemically stable non-reducible modification is preferred. For example, phosphorothioate
30 modifications can also be used.

The expressed ribozyme RNA can be isolated from bacterial or eucaryotic cells by routine procedures such as lysis followed by guanidine isothiocyanate isolation.

The current known self-cleaving site of *Tetrahymena* can be used in
35 an alternative vector of this invention. If desired, the full-length

Tetrahymena sequence may be used, or a shorter sequence may be used. It is preferred that, in order to decrease the superfluous sequences in the self-cleaving site at the 5' cleavage end, the hairpin normally present in the *Tetrahymena* ribozyme should contain the therapeutic second ribozyme 3' sequence and its complement. That is, the first releasing ribozyme-encoding DNA is provided in two portions, separated by DNA encoding the desired second ribozyme. For example, if the therapeutic second ribozyme recognition sequence is CGGACGA/CGAGGA, then CGAGGA is provided in the self-cleaving site loop such that it is in a stem structure recognized by the *Tetrahymena* ribozyme. The loop of the stem may include a restriction endonuclease site into which the desired second ribozyme-encoding DNA is placed.

If desired, the vector may be used in a therapeutic protocol by use of the systems described by Lechner, PCT WO 92/13070, hereby incorporated by reference herein, to allow a timed expression of the therapeutic second ribozyme, as well as an appropriate shut off of cell or gene function. Thus, the vector will include a promoter which appropriately expresses enzymatically active RNA only in the presence of an RNA or another molecule which indicates the presence of an undesired organism or state. Such enzymatically active RNA will then kill or harm the cell in which it exists, as described by Lechner, *id.*, or act to cause reduced expression of a desired protein product.

A number of suitable RNA vectors may also be used in this invention. The vectors include plant viroids, plant viruses which contain single or double-stranded RNA genomes and animal viruses which contain RNA genomes, such as the picornaviruses, myxoviruses, paramyxoviruses, hepatitis A virus, reovirus and retroviruses. In many instances cited, use of these viral vectors also results in tissue specific delivery of the ribozymes.

Example 21: Design of self-processing cassettes

In a preferred embodiment, applicant compared the *in vitro* and *in vivo* cis-cleaving activity of three different ribozyme motifs—the hammerhead, the hairpin and the hepatitis delta virus ribozyme—in order to assess their potential to process the ends of transcripts *in vivo*. To make a direct comparison among the three, however, it is important to design the ribozyme-containing transcripts to be as similar as possible. To this end,

- all the ribozyme cassettes contained the same trans-acting hammerhead ribozyme followed immediately by one of the three cis-acting ribozymes (Figure 23-25). For simplicity, applicant refers to each cassette by an abbreviation that indicates the downstream cis-cleaving ribozyme only.
- 5 Thus HH refers to the cis-cleaving cassette containing a hammerhead ribozyme, while HP and HDV refer to the cassettes containing hairpin and hepatitis delta virus cis-cleaving ribozymes, respectively. The general design of the ribozyme cassettes, as well as specific differences among the cassettes, are outlined below.
- 10 A sequence predicted to form a stable stem-loop structure is included at the 5' end of all the transcripts. The hairpin stem contains the T7 RNA polymerase initiation sequence (Milligan & Uhlenbeck, 1989 Methods Enzymol. 180, 51) and its complement, separated by a stable tetra-loop (Antao et al., 1991 Nucleic Acids Res. 19, 5901). By incorporating the T7 15 initiation sequence into a stem-loop structure, applicant hoped to avoid nonproductive base pairing interactions with either the trans-acting ribozyme or with the cis-acting ribozyme. The presence of a hairpin at the end of a transcript may also contribute to the stability of the transcript *in vivo*. These are non-limiting examples. Those in the art will recognize that 20 other embodiments can be readily generated using a variety of promoters, initiator sequences and stem-loop structure combinations generally known in the art.
- The trans-acting ribozyme used in this study is targeted to a site B (5'...CUGGAGUCGACCUUC...3'). The 5' binding arm of the ribozyme, 5'-GAAGGUC-3', and the core of the ribozyme, 5'-CUGAUGAGGCCGAAAGGCCGAA-3', remain constant in all cases. In addition, all transcripts also contain a single nucleotide between the 5' 25 stem-loop and the first nucleotide of the ribozyme. The linker nucleotide was required to obtain the same activity *in vitro* that was measured with an identical ribozyme lacking the 5' hairpin. Because the three cis-cleaving 30 ribozymes have different requirements at the site of cleavage, slight differences were unavoidable at the 3' end of the processed transcript. The junction between the trans- and cis-acting ribozyme is, however, designed so that there is minimal extraneous sequence left at the 3' end of the trans- 35 cleaving ribozyme once cis-cleavage occurs. The only differences between the constructs lie in the 3' binding arm of the ribozyme, where

either 6 or 7 nucleotides, 5'-ACUCCA(+/-G)-3', complementary to the target sequence are present and where, after processing, two to five extra nucleotides remain.

The cis-cleaving hammerhead ribozyme used in the HH cassette is
5 based on the design of Grosshans and Cech, 1991 supra. As shown in
Figure 23, the 3' binding arm of the trans-acting ribozyme is included in the
required base-pairing interactions of the cis-cleaving ribozyme to form stem
I. Two extra nucleotides, UC, were included at the end of the 3' binding
10 arm to form the self-processing hammerhead ribozyme site (Ruffner et al.,
1990 supra) which remain on the 3' end of the trans-acting ribozyme
following self-processing.

The hairpin ribozyme portion of the HP self-processing construct is
based on the minimal wild-type sequence (Hampel & Tritz, 1989 supra). A
tetra-loop at the end of helix 1 (3' side of the cleavage site) serves to link
15 the two portions and thus allows a minimal five nucleotides to remain at the
end of the released trans-acting ribozyme following self-processing. Two
variants of HP were designed: HP(GU) and HP(GC). The HP(GU) was
constructed with a G·U wobble base pair in helix 2 (A52G substitution;
Figure 24). This slight destabilization of helix 2 was intended to improve
20 self-processing activity by promoting product release and preventing the
reverse reaction (Berzal-Herranz et al., 1992 Genes & Dev. 6, 129;
Chowrira et al., 1993 Biochemistry 32, 1088). The HP(GC) cassette was
constructed as a control for strong base-pairing interactions in helix 2
(U77C and A52G substitution; Figure 24). Another modification to
25 discourage the reverse ligation reaction of the hairpin ribozyme was to
shorten helix 1 (Figure 24) by one base pair relative to the wild-type
sequence (Chowrira & Burke, 1991 Biochemistry 30, 8518).

The HDV ribozyme self-processes efficiently when the nucleotide 5' to
the cleavage site is a pyrimidine, and somewhat less so when adenosine is
30 in that position. No other sequence requirements have been identified
upstream of the cleavage site, however, we have observed some decrease
in activity when a stem-loop structure was present within 2 nt of the
cleavage site. The HDV self-processing construct (Fig 25) was designed to
generate the trans-acting hammerhead ribozyme with only two additional
35 nucleotides at its 3' end after self-processing. The HDV sequence used
here is based on the anti-genomic sequence (Perrotta & Been, 1992 supra)

but includes the modifications of Been et al., 1992 (Biochemistry 31, 11843) in which cis-cleavage activity of the ribozyme was improved by the substitution of a shortened helix 4 for a wild-type stem-loop (Figure 25).

- To prepare DNA inserts that encode self-processing ribozyme cassettes, partially overlapping top- and bottom-strand oligonucleotides (60-90 nucleotides) were designed to include sequences for the T7 promoter, the trans-acting ribozyme, the cis-cleaving ribozyme and appropriate restriction sites for use in cloning (see Fig. 26). The single-strand portions of annealed oligonucleotides were converted to double-strands using Sequenase® (U.S. Biochemicals). Insert DNA was ligated into *EcoR1/HindIII*-digested puc18 and transformed into *E. coli* strain DH5 α using standard protocols (Maniatis et al., 1982 in Molecular Cloning Cold Spring Harbor Press). The identity of positive clones was confirmed by sequencing small-scale plasmid preparations.
- 15 Larger scale preparations of plasmid DNA for use as *in vitro* transcription templates and in transactions were prepared using the protocol and columns from QIAGEN Inc. (Studio City, CA) except that an additional ethanol precipitation was included as the final step.

Example 22: RNA Processing *in vitro*

- 20 Transcription reactions containing linear plasmid templates were carried out essentially as described (Milligan & Uhlenbeck, 1989 Supra; Chowrira & Burke, 1991 Supra). In order to prepare 5' end-labeled transcripts, standard transcription reactions were carried out in the presence of 10-20 μ Ci [γ -32P]GTP, 200 μ M each NTP and 0.5 to 1 μ g of 25 linearized plasmid template. The concentration of MgCl₂ was maintained at 10 mM above the total nucleotide concentration.

To compare the ability of the different ribozyme cassettes to self-process *in vitro*, each construct was transcribed and allowed to undergo self-processing under identical conditions at 37°C. For these comparisons, 30 equal amounts of linearized DNA templates bearing the various ribozyme cassettes were transcribed in the presence of [γ -32P]GTP to generate 5' end-labeled transcripts. In this manner only the full-length, unprocessed transcripts and the released trans-ribozymes are visualized by autoradiography. In all reactions, Mg²⁺ was included at 10 mM above the 35 nucleotide concentration so that cleavage by all the ribozyme cassettes

would be supported. Transcription templates were linearized at several positions by digestion with different restriction enzymes so that self-processing in the presence of increasing lengths of downstream sequence could be compared (see Fig. 26). The resulting transcripts have either 4-5
5 non-ribozyme nucleotides at the 3' end (*HindIII*-digested template), 220 nucleotides (*NdeI* digested templates) or 454 nucleotides of downstream sequence (*RcaI* digested template).

As shown in Figure 27, all four ribozyme cassettes are capable of self-processing and yield RNA products of expected sizes. Two nucleotides
10 essential for hammerhead ribozyme activity (Ruffner et al., 1990 supra) have been changed in the HH(mutant) core sequence (see Figure 23) and so this transcript is unable to undergo self-processing (Fig. 27). This is evidenced by the lack of a released 5' RNA in the HH(mutant), although the full-length RNAs are present. Comparison of the amounts of released
15 trans-ribozyme (Fig. 27) indicate that there are differences in the ability of these ribozymes to self-process *in vitro*, especially with respect to the presence of downstream sequence. For the two HP constructs, it is clear that HP(GC) is more efficient than the HP(GU) ribozyme, both in the presence and in the absence of extra downstream sequence. In addition,
20 the activity of HP(GU) falls off more dramatically when downstream sequence is present. The stronger G:C base pair likely contributes to the HP(GC) construct's ability to fold correctly (and/or more quickly) into the productive structure, even when as much as 216 extra nucleotides are present downstream. The HH ribozyme construct is also quite efficient at
25 self-processing, and slightly better than the HP(GU) construct even when downstream sequence is present.

Of the three ribozyme motifs, the presence of extra downstream sequence seems to most affect the efficiency of HDV. When no extra sequence is present downstream, HDV is quite efficient and self-processes
30 to approximately the same level as the HH and HP(GC) cassettes. However, when extra downstream sequence is present, the self-processing activity seems to decrease almost as dramatically as is seen with the (sub-optimal) HP(GU) cassette.

Example 23: Kinetics of self-processing reaction

*Hind*III-digested template (250 ng) was used in a standard transcription reaction mixture containing: 50 mM Tris-HCl pH 8.3; 1 mM ATP, GTP and UTP; 50 µM CTP; 40 µCi [α -³²P]CTP; 12 mM MgCl₂; 10 mM DTT. The transcription/self-processing reaction was initiated by the addition of T7 RNA polymerase (15 U/µl). Aliquots of 5 µl were taken at regular time intervals and the reaction was stopped by adding an equal volume of 2x formamide loading buffer (95% formamide, 15 mM EDTA, & dyes) and freezing on dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel and results were quantitated by PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Ribozyme self-cleavage rates were determined from non-linear, least-squares fits (KaleidaGraph, Synergy Software, Reeding, PA) of the data to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt} (1 - e^{-kt})$$

where t represents time and k represents the unimolecular rate constant for cleavage (Long & Uhlenbeck, 1994 Proc. Natl. Acad. Sci. USA 91, 6977).

Linear templates were prepared by digesting the plasmids with *Hind*III so that transcripts will contain only four to five vector-derived nucleotides at the 3' end (see Figure 23-25). By comparison of the unimolecular rate constant (k) determined for each construct, it is clear that HH is the most efficient at self-processing (Table 44). The HH transcript self-processes 2-fold faster than HDV and 3-fold faster than HP(GC) transcripts. Although the HP(GU) RNA undergoes self-processing, it is at least 6-fold slower than the HP(GC) construct. This is consistent with previous observations that the stability of helix 2 is essential for self-processing and trans-cleavage activity of the hairpin ribozyme (Hampel et al., 1990 supra; Chowrira & Burke, 1991 supra). The rate of HH self-cleavage during transcription measured here (1.2 min⁻¹) is similar to the rate measured by Long and Uhlenbeck 1994 supra using a HH that has a different stem I and stem III. Self-processing rates during transcription for HP and HDV have not been previously reported. However, self-processing of the HDV ribozyme—as measured here during transcription—is significantly slower than when tested after isolation from a denaturing gel (Been et al., 1992 supra). This decrease likely reflects the difference in protocol as well as the presence of 5' flanking sequence in the HDV construct used here.

Example 24: Effect of downstream sequences on trans-cleavage *in vitro*

Transcripts containing the trans ribozyme with or without 3' flanking sequences were assayed for their ability to cleave their target in trans. To this end, transcripts from three templates were resolved on a preparative gel and bands corresponding both to processed trans-acting ribozymes from the HH transcription reaction, and to full-length HH(mutant) and ΔHDV transcripts were isolated. In all three transcripts the trans-acting ribozyme portion is identical—with the exception of sequences at their 3' ends. The HH trans-acting ribozyme contains only an additional UC at its 3' end, while HH(mutant) and ΔHDV have 52 and 37 nucleotides, respectively, at their 3' ends. A 622 nucleotide, internally-labeled target RNA was incubated, under ribozyme excess conditions, along with the three ribozyme transcripts in a standard reaction buffer.

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 622 nt region (containing hammerhead site P) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α -32P]CTP (Chowrira & Burke, 1991 *supra*). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1 μ M) and internally labeled 622 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager® (Molecular Dynamics, Sunnyvale, CA).

The HH trans-acting ribozyme cleaves the target RNA approximately 10-fold faster than the ΔHDV transcript and greater than 20-fold faster than

the HH(mutant) transcript (Figure 28). The additional nucleotides at the end of HH(mutant) form 7 base-pairs with the 3' target-binding arm of the trans-acting ribozyme (Figure 23). This interaction must be disrupted (at a cost of 6 kcal/mole) to make the trans-acting ribozyme available for binding
5 the target sequence. In contrast, the additional nucleotides at the end of ΔHDV were not designed to form any strong, alternative base-pairing with the trans-ribozyme. Nevertheless, the ΔHDV sequences are predicted to form multiple structures involving the 3' target-binding arm of the trans
10 ribozyme that have stabilities ranging from 1-2 kcal/mole. Thus, the observed reductions in activity for the ΔHDV and HH(mutant) constructs are consistent with the predicted folded structures, and it reinforces the view that the flanking sequences can decrease the catalytic efficiency of a ribozyme through nonproductive interactions with either the ribozyme or the substrate or both.

15 Example 25: RNA self-processing *in vivo*

Since three of the constructs (HH, HDV and HP(GC)) self-process efficiently in solution, the affect of the mammalian cellular milieu on ribozyme self-processing was next explored by applicant. A transient expression system was employed to investigate ribozyme activity *in vivo*. A
20 mouse cell line (OST7-1) that constitutively expresses T7 RNA polymerase in the cytoplasm was chosen for this study (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. USA 87, 6743). In these cells plasmids containing a ribozyme cassette downstream of the T7 promoter will be transcribed efficiently in the cytoplasm (Elroy-Stein & Moss, 1990 supra).

25 Monolayers of a mouse L9 fibroblast cell line (OST7-1; Elroy-Stein and Moss, 1990 supra) were grown in 6-well plates with ~ 5x10⁵ cells/well. Cells were transfected with circular plasmids (5 µg/well) using the calcium phosphate-DNA precipitation method (Maniatis et al., 1982 supra). Cells were lysed (4 hours post-transfection) by the addition of standard lysis
30 buffer (200 µl/well) containing 4M guanadinium isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarkosyl (Chomczynski and Sacchi, 1987 Anal. Biochem. 162, 156), and 50 mM EDTA pH 8.0. The lysate was extracted once with water-saturated phenol followed by one extraction with chloroform:isoamyl alcohol (25:1). Total cellular RNA was precipitated with
35 an equal volume of isopropanol. The RNA pellet was resuspended in 0.2

M ammonium acetate and reprecipitated with ethanol. The pellet was then washed with 70% ethanol and resuspended in DEPC-treated water.

Purified cellular RNA (3 µg/reaction) was first denatured in the presence of a 5' end-labeled DNA primer (100 pmol) by heating to 90°C for
5 2 min. in the absence of Mg²⁺, and then snap-cooling on ice for at least 15 min. This protocol allows for efficient annealing of the primer to its complementary RNA sequence. The primer was extended using Superscript II reverse transcriptase (8 U/µl; BRL) in a buffer containing 50 mM Tris-HCl pH 8.3; 10 mM DTT; 75 mM KCl; 1 mM MgCl₂; 1 mM each
10 dNTP. The extension reaction was carried out at 42°C for 10 min. The reaction was terminated by adding an equal volume of 2x formamide gel loading buffer and freezing on crushed dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel. The primer sequences are as follows: HH primer, 5'-CTCCAGTTCGAGCTT-3'; HDV primer, 5'-
15 AAGTAGCCCAGGTCGGACC-3'; HP primer, 5'-ACCAGGTAATATACCACAAAC-3'.

As shown in Figure 29, specific bands corresponding to full-length precursor RNA and 3' cleavage products were detected from cells transfected with the self-processing cassettes. All three constructs, in
20 addition to being transcriptionally active, appear to self-process efficiently in the cytoplasm of OST7-1 cells. In particular, the HH and HP(GC) constructs self-process to greater than 95%. The overall extent of self-processing in OST7-1 cells appears to be strikingly similar to the extent of self-processing *in vitro* (Figure 29 "In Vitro +MgCl₂" vs. "Cellular").

25 Consistent with the *in vitro* self-processing results, the HP(GU) cassette self-processed to approximately 50% in OST7-1 cells. As expected, transfection with plasmids containing the HH(mutant) cassette yielded a primer-extension product corresponding to the full-length RNA with no detectable cleavage products (Figure 29). The latter result strongly
30 suggests that the primer extension band corresponding to the 3' cleavage product is not an artifact of reverse transcription.

Applicant was concerned with the possibility that RNA self-processing might occur during cell lysis, RNA isolation and /or the primer extension assay. Two precautions were taken to exclude this possibility. First, 50 mM EDTA was included in the lysis buffer. EDTA is a strong chelator of divalent
35

metal ions such as Mg²⁺ and Ca²⁺ that are necessary for ribozyme activity. Divalent metal ions are therefore unavailable to self-processing RNAs following cell lysis. A second precaution involved using primers in the primer-extension assay that were designed to hybridize to essential regions of the processing ribozyme. Binding of these primers should prevent the 3' cis-acting ribozymes from folding into the conformation essential for catalytic activity.

Two experiments were carried out to further eliminate the possibility that self-processing is occurring either during RNA preparations or during the primer extension analysis. The first experiment involves primer extension analysis on full-length precursor RNAs that were added to non-transfected OST7-1 lysates after cell lysis. Thus, only if self-processing is occurring at some point after lysis would cleavage products be detected. Full-length precursor RNAs were prepared by transcribing under conditions of low Mg²⁺ (5 mM) and high NTP concentration (total 12 mM) in an attempt to eliminate the free Mg²⁺ required for the self-processing reaction (Michel et al. 1992 *Genes & Dev.* 6, 1373). The full-length precursor RNAs were gel-purified, and a known amount was added to lysates of non-transfected OST7-1 cells. RNA was purified from these lysates and incubated for 1 hr in DEPC-treated water at 37° C prior to the standard primer extension analysis (Figure 29, *in vitro* "-MgCl₂" control). The predominant RNA detected in all cases corresponds to the primer extension product of full-length precursor RNAs. If, instead, the purified RNA containing the full-length precursor is incubated in 10 mM MgCl₂ prior to the primer extension analysis, most or all of the RNA detected by primer extension analysis undergoes cleavage (Figure 29, *in vitro* "+MgCl₂" control). These results indicate that the standard RNA isolation and primer extension protocols used here do not provide a favorable environment for RNA self-processing, even though the RNA in question is inherently able to undergo self-cleavage.

In a second experiment to demonstrate lack of self-processing during work up, internally-labeled precursor RNAs were prepared and added to non-transfected OST7-1 lysates as in the previous control. The internally-labeled precursor RNAs were carried through the RNA purification and primer extension reactions (in the presence of unlabeled primers) and analyzed to determine the extent of self-processing. By this analysis, the

vast majority of the added full-length RNA remained intact during the entire process of RNA isolation and primer extension.

These two control experiments validate the protocols used and support applicant's conclusion that the self-processing reactions catalyzed
5 by HH, HDV and HP(GC) cassettes are occurring in the cytoplasm of OST7-1 cells.

Sequences in figures 23 through 25 are meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art.

10 In addition, those in the art will recognize that Applicant provides guidance through the above examples as to how to best design vectors of this invention so that secondary structure of the mRNA allows efficient cleavage by releasing ribozymes. Thus, the specific constructs are not limiting in this invention. Such constructs can be readily tested as
15 described above for such secondary structure, either by computer folding algorithms or empirically. Such constructs will then allow at least 80% completion of release of ribozymes, which can be readily determined as described above or by methods known in the art. That is, any such secondary structure in the RNA does not reduce release of the ribozymes
20 by more than 20%.

IV. Ribozymes Expressed by RNA Polymerase III

Applicant has determined that the level of production of a foreign RNA, using a RNA polymerase III (pol III) based system, can be significantly enhanced by ensuring that the RNA is produced with the 5' terminus and a
25 3' region of the RNA molecule base-paired together to form a stable intramolecular stem structure. This stem structure is formed by hydrogen bond interactions (either Watson-Crick or non-Watson-Crick) between nucleotides in the 3' region (at least 8 bases) and complementary nucleotides in the 5' terminus of the same RNA molecule.

30 Although the example provided below involves a type 2 pol III gene unit, a number of other pol III promoter systems can also be used, for example, tRNA (Hall et al., 1982 *Cell* 29, 3-5), 5S RNA (Nielsen et al., 1993, *Nucleic Acids Res.* 21, 3631-3636), adenovirus VA RNA (Fowlkes and Shenk, 1980 *Cell* 22, 405-413), U6 snRNA (Gupta and Reddy, 1990

Nucleic Acids Res. 19, 2073-2075), vault RNA (Kickofer et al., 1993 *J. Biol. Chem.* 268, 7868-7873), telomerase RNA (Romero and Blackburn, 1991 *Cell* 67, 343-353), and others.

The construct described in this invention is able to accumulate RNA to
5 a significantly higher level than other constructs, even those in which 5'
and 3' ends are involved in hairpin loops. Using such a construct the level
of expression of a foreign RNA can be increased to between 20,000 and
10 50,000 copies per cell. This makes such constructs, and the vectors
encoding such constructs, excellent for use in decoy, therapeutic editing
15 and antisense protocols as well as for ribozyme formation. In addition, the
molecules can be used as agonist or antagonist RNAs (affinity RNAs).
Generally, applicant believes that the intramolecular base-paired
interaction between the 5' terminus and the 3' region of the RNA should be
in a double-stranded structure in order to achieve enhanced RNA
15 accumulation.

Thus, in one preferred embodiment the invention features a pol III
promoter system (*e.g.*, a type 2 system) used to synthesize a chimeric RNA
molecule which includes tRNA sequences and a desired RNA (*e.g.*, a
tRNA-based molecule).

20 The following exemplifies this invention with a type 2 pol III promoter
and a tRNA gene. Specifically to illustrate the broad invention, the RNA
molecule in the following example has an A box and a B box of the type 2
pol III promoter system and has a 5' terminus or region able to base-pair
25 with at least 8 bases of a complementary 3' end or region of the same RNA
molecule. This is meant to be a specific example. Those in the art will
recognize that this is but one example, and other embodiments can be
readily generated using other pol III promoter systems and techniques
generally known in the art.

30 By "terminus" is meant the terminal bases of an RNA molecule, ending
in a 3' hydroxyl or 5' phosphate or 5' cap moiety. By "region" is meant a
stretch of bases 5' or 3' from the terminus that are involved in base-paired
interactions. It need not be adjacent to the end of the RNA. Applicant has
determined that base pairing of at least one end of the RNA molecule with
a region not more than about 50 bases, and preferably only 20 bases, from

the other end of the molecule provides a useful molecule able to be expressed at high levels.

By "3' region" is meant a stretch of bases 3' from the terminus that are involved in intramolecular base-paired interaction with complementary 5 nucleotides in the 5' terminus of the same molecule. The 3' region can be designed to include the 3' terminus. The 3' region therefore is ≥ 0 10 nucleotides from the 3' terminus. For example, in the S35 construct described in the present invention (Fig. 40) the 3' region is one nucleotide from the 3' terminus. In another example, the 3' region is ~ 43 nt from 3' 15 terminus. These examples are not meant to be limiting. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. Generally, it is preferred to have the 3' region within 100 bases of the 3' terminus.

By "tRNA molecule" is meant a type 2 pol III driven RNA molecule that 15 is generally derived from any recognized tRNA gene. Those in the art will recognize that DNA encoding such molecules is readily available and can be modified as desired to alter one or more bases within the DNA encoding the RNA molecule and/or the promoter system. Generally, but not always, such molecules include an A box and a B box that consist of sequences 20 which are well known in the art (and examples of which can be found throughout the literature). These A and B boxes have a certain consensus sequence which is essential for optimal pol III transcription.

By "chimeric tRNA molecule" is meant a RNA molecule that includes a 25 pol III promoter (type 2) region. A chimeric tRNA molecule, for example, might contain an intramolecular base-paired structure between the 3' region and complementary 5' terminus of the molecule, and includes a foreign RNA sequence at any location within the molecule which does not affect the activity of the type 2 pol III promoter boxes. Thus, such a foreign RNA may be provided at the 3' end of the B box, or may be provided in 30 between the A and the B box, with the B box moved to an appropriate location either within the foreign RNA or another location such that it is effective to provide pol III transcription. In one example, the RNA molecule may include a hammerhead ribozyme with the B box of a type 2 pol III promoter provided in stem II of the ribozyme. In a second example, the B 35 box may be provided in stem IV region of a hairpin ribozyme. A specific example of such RNA molecules is provided below. Those in the art will

recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "desired RNA" molecule is meant any foreign RNA molecule which is useful from a therapeutic, diagnostic, or other viewpoint. Such 5 molecules include antisense RNA molecules, decoy RNA molecules, enzymatic RNA, therapeutic editing RNA and agonist and antagonist RNA.

By "antisense RNA" is meant a non-enzymatic RNA molecule that binds to another RNA (target RNA) by means of RNA-RNA interactions and alters the activity of the target RNA (Eguchi et al., 1991 *Annu. Rev. 10 Biochem.* 60, 631-652). By "enzymatic RNA" is meant an RNA molecule with enzymatic activity (Cech, 1988 *J.American. Med. Assoc.* 260, 3030-3035). Enzymatic nucleic acids (ribozymes) act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic 15 portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA.

By "decoy RNA" is meant an RNA molecule that mimics the natural 20 binding domain for a ligand. The decoy RNA therefore competes with natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV *tat* protein, thereby preventing it from binding to TAR sequences encoded in the HIV 25 RNA (Sullenger et al., 1990 *Cell* 63, 601-608). This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "therapeutic editing RNA" is meant an antisense RNA that can bind 30 to its cellular target (RNA or DNA) and mediate the modification of a specific base.

By "agonist RNA" is meant an RNA molecule that can bind to protein receptors with high affinity and cause the stimulation of specific cellular pathways.

By "antagonist RNA" is meant an RNA molecule that can bind to cellular proteins and prevent it from performing its normal biological function (for example, see Tsai et al., 1992 *Proc. Natl. Acad. Sci. USA* 89, 8864-8868).

- 5 In other aspects, the invention includes vectors encoding RNA molecules as described above, cells including such vectors, methods for producing the desired RNA, and use of the vectors and cells to produce this RNA.

Thus, the invention features a transcribed non-naturally occurring RNA
10 molecule which includes a desired therapeutic RNA portion and an intramolecular stem formed by base-pairing interactions between a 3' region and complementary nucleotides at the 5' terminus in the RNA. The stem preferably includes at least 8 base pairs, but may have more, for example, 15 or 16 base pairs.

- 15 In preferred embodiments, the 5' terminus of the chimeric tRNA includes a portion of the precursor molecule of the primary tRNA molecule, of which ≥ 8 nucleotides are involved in base-pairing interaction with the 3' region; the chimeric tRNA contains A and B boxes; natural sequences 3' of the B box are deleted, which prevents endogenous RNA processing; the
20 desired RNA molecule is at the 3' end of the B box; the desired RNA molecule is between the A and the B box; the desired RNA molecule includes the B box; the desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA; the molecule has an
25 intramolecular stem resulting from a base-paired interaction between the 5' terminus of the RNA and a complementary 3' region within the same RNA, and includes at least 8 bases; and the 5' terminus is able to base pair with at least 15 bases of the 3' region.

- 30 In most preferred embodiments, the molecule is transcribed by a RNA polymerase III based promoter system, e.g., a type 2 pol III promoter system; the molecule is a chimeric tRNA, and may have the A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases; DNA vector encoding the RNA molecule of claim 51..

In other related aspects, the invention features an RNA or DNA vector encoding the above RNA molecule, with the portions of the vector encoding the RNA functioning as a RNA pol III promoter; or a cell containing the vector ; or a method to provide a desired RNA molecule in a cell, by 5 introducing the molecule into a cell with an RNA molecule as described above. The cells can be derived from animals, plants or human beings.

In order for RNA-based gene therapy approaches to be effective, sufficient amounts of the therapeutic RNA must accumulate in the appropriate intracellular compartment of the treated cells. Accumulation is 10 a function of both promoter strength of the antiviral gene, and the intracellular stability of the antiviral RNA. Both RNA polymerase II (pol II) and RNA polymerase III (pol III) based expression systems have been used to produce therapeutic RNAs in cells (Sarver & Rossi, 1993 *AIDS Res. & Human Retroviruses* 9, 483-487; Yu et al., 1993 *P.N.A.S.(USA)* 90, 6340-15 6344). However, pol III based expression cassettes are theoretically more attractive for use in expressing antiviral RNAs for the following reasons. Pol II produces messenger RNAs located exclusively in the cytoplasm, whereas pol III produces functional RNAs found in both the nucleus and the cytoplasm. Pol II promoters tend to be more tissue restricted, whereas pol 20 III genes encode tRNAs and other functional RNAs necessary for basic "housekeeping" functions in all cell types. Therefore, pol III promoters are likely to be expressed in all tissue types. Finally, pol III transcripts from a given gene accumulate to much greater levels in cells relative to pol II genes.

25 Intracellular accumulation of therapeutic RNAs is also dependent on the method of gene transfer used. For example, the retroviral vectors presently used to accomplish stable gene transfer, integrate randomly into the genome of target cells. This random integration leads to varied expression of the transferred gene in individual cells comprising the bulk 30 treated cell population. Therefore, for maximum effectiveness, the transferred gene must have the capacity to express therapeutic amounts of the antiviral RNA in the entire treated cell population, regardless of the integration site.

Pol III System

The following is just one non-limiting example of the invention. A pol III based genetic element derived from a human tRNA_imet gene and termed Δ3-5 (Fig. 33; Adeniyi-Jones et al., 1984 *supra*), has been adapted 5 to express antiviral RNAs (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523). This element was inserted into the DC retroviral vector (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523) to accomplish stable gene transfer, and used to express antisense RNAs against moloney murine leukemia virus and anti-HIV decoy RNAs (Sullenger et al., 1990 *Mol. Cell. 10, Biol.* 6512-6523; Sullenger et al., 1990 *Cell* 63, 601-608; Sullenger et 15 al., 1991 *J. Virol.* 65, 6811-6816; Lee et al., 1992 *The New Biologist* 4, 66-74). Clonal lines are expanded from individual cells present in the bulk population, and therefore express similar amounts of the therapeutic RNA in all cells. Development of a vector system that generates therapeutic 15 levels of therapeutic RNA in all treated cells would represent a significant advancement in RNA based gene therapy modalities.

Applicant examined hammerhead (HHI) ribozyme (RNA with enzymatic activity) expression in human T cell lines using the Δ3-5 vector system (These constructs are termed "Δ3-5/HHI"; Fig. 34). On average, 20 ribozymes were found to accumulate to less than 100 copies per cell in the bulk T cell populations. In an attempt to improve expression levels of the Δ3-5 chimera, the applicant made a series of modified Δ3-5 gene units containing enhanced promoter elements to increase transcription rates, and inserted structural elements to improve the intracellular stability of the 25 ribozyme transcripts (Fig. 34). One of these modified gene units, termed S35, gave rise to more than a 100-fold increase in ribozyme accumulation in bulk T cell populations relative to the original Δ3-5/HHI vector system. Ribozyme accumulation in individual clonal lines from the pooled T cell populations ranged from 10 to greater than 100 fold more than those 30 achieved with the original Δ3-5/HHI version of this vector.

The S35 gene unit may be used to express other therapeutic RNAs including, but not limited to, ribozymes, antisense, decoy, therapeutic editing, agonist and antagonist RNAs. Application of the S35 gene unit would not be limited to antiviral therapies, but also to other diseases, such 35 as cancer, in which therapeutic RNAs may be effective. The S35 gene unit may be used in the context of other vector systems besides retroviral

vectors, including but not limited to, other stable gene transfer systems such as adeno-associated virus (AAV; Carter, 1992 *Curr. Opin. Genet. Dev.* 3, 74), as well as transient vector systems such as plasmid delivery and adenoviral vectors (Berkner, 1988 *BioTechniques* 6, 616-629).

5 As described below, the S35 vector encodes a truncated version of a tRNA wherein the 3' region of the RNA is base-paired to complementary nucleotides at the 5' terminus, which includes the 5' precursor portion that is normally processed off during tRNA maturation. Without being bound by any theory, Applicant believes this feature is important in the level of
10 expression observed. Thus, those in the art can now design equivalent RNA molecules with such high expression levels. Below are provided examples of the methodology by which such vectors and tRNA molecules can be made.

Δ3-5 Vectors

15 The use of a truncated human tRNA_i^{met} gene, termed Δ3-5 (*Fig. 33*; Adeniyi-Jones et al., 1984 *supra*), to drive expression of antisense RNAs, and subsequently decoy RNAs (Sullenger et al., 1990 *supra*) has recently been reported. Because tRNA genes utilize internal pol III promoters, the antisense and decoy RNA sequences were expressed as chimeras
20 containing tRNA_i^{met} sequences. The truncated tRNA genes were placed into the U3 region of the 3' moloney murine leukemia virus vector LTR (Sullenger et al., 1990 *supra*).

Base-Paired Structures

25 Since the Δ3-5 vector combination has been successfully used to express inhibitory levels of both antisense and decoy RNAs, applicant cloned ribozyme-encoding sequences (termed as "Δ3-5/HHI") into this vector to explore its utility for expressing therapeutic ribozymes. However, low ribozyme accumulation in human T cell lines stably transduced with this vector was observed (*Fig. 35*). To try and improve accumulation of the
30 ribozyme, applicant incorporated various RNA structural elements (*Fig. 34*) into one of the ribozyme chimeras (Δ3-5/HHI).

Two strategies were used to try and protect the termini of the chimeric transcripts from exonucleolytic degradation. One strategy involved the incorporation of stem-loop structures into the termini of the transcript. Two

such constructs were cloned, S3 which contains a stem-loop structure at the 3' end, and S5 which contains stem-loop structures at both ends of the transcript (Figure 34). The second strategy involved modification of the 3' terminal sequences such that the 5' terminus and the 3' end sequences
5 can form a stable base-paired stem. Two such constructs were made: S35 in which the 3' end was altered to hybridize to the 5' leader and acceptor stem of the tRNA_imet domain, and S35Plus which was identical to S35 but included more extensive structure formation within the non-ribozyme portion of the Δ3-5 chimeras (Figure 34). These stem-loop structures are
10 also intended to sequester non-ribozyme sequences in structures that will prevent them from interfering with the catalytic activity of the ribozyme. These constructs were cloned, producer cell lines were generated, and stably-transduced human MT2 (Harada et al., 1985 *supra*) and CEM (Nara & Fischinger, 1988 *supra*) cell lines were established (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). The RNA sequences and
15 structure of S35 and S35 Plus are provided in Figures 40-47.

Referring to Figure 48, there is provided a general structure for a chimeric RNA molecule of this invention. Each N independently represents none or a number of bases which may or may not be base paired. The A and B boxes are optional and can be any known A or B box, or a consensus sequence as exemplified in the figure. The desired nucleic acid to be expressed can be any location in the molecule, but preferably is on those places shown adjacent to or between the A and B boxes (designated by arrows). Figure 49 shows one example of such a structure in which a desired RNA is provided 3' of the intramolecular stem. A specific example of such a construct is provided in Figures 50 and 51.

Example 26: Cloning of Δ3-5-Ribozyme Chimera

Oligonucleotides encoding the S35 insert that overlap by at least 15 nucleotides were designed (5' GATCCACTCTGCTGTTCTGTTTGA 3'
30 and 5' CGCGTCAAAACAGAACAGCAGAGTG 3'). The oligonucleotides (10 μM each) were denatured by boiling for 5 min in a buffer containing 40 mM Tris.HCl, pH8.0. The oligonucleotides were allowed to anneal by snap cooling on ice for 10-15 min.

The annealed oligonucleotide mixture was converted into a double-stranded molecule using Sequenase® enzyme (US Biochemicals) in a
35

buffer containing 40 mM Tris.HCl, pH7.5, 20 mM MgCl₂, 50 mM NaCl, 0.5 mM each of the four deoxyribonucleotide triphosphates, 10 mM DTT. The reaction was allowed to proceed at 37°C for 30 min. The reaction was stopped by heating to 70°C for 15 min.

- 5 The double stranded DNA was digested with appropriate restriction endonucleases (*Bam*H_I and *M*_I*u*_I) to generate ends that were suitable for cloning into the Δ3-5 vector.

- 10 The double-stranded insert DNA was ligated to the Δ3-5 vector DNA by incubating at room temperature (about 20°C) for 60 min in a buffer containing 66 mM Tris.HCl, pH 7.6, 6.6 mM MgCl₂, 10 mM DTT, 0.066 μM ATP and 0.1U/μl T4 DNA Ligase (US Biochemicals).

- 15 Competent *E. coli* bacterial strain was transformed with the recombinant vector DNA by mixing the cells and DNA on ice for 60 min. The mixture was heat-shocked by heating to 37°C for 1 min. The reaction mixture was diluted with LB media and the cells were allowed to recover for 60 min at 37°C. The cells were plated on LB agar plates and incubated at 37°C for ~ 18 h.

- 20 Plasmid DNA was isolated from an overnight culture of recombinant clones using standard protocols (Ausubel et al., *Curr. Protocols Mol. Biology* 1990, Wiley & Sons, NY).

The identity of the clones were determined by sequencing the plasmid DNA using the Sequenase® DNA sequencing kit (US Biochemicals).

- 25 The resulting recombinant Δ3-5 vector contains the S35 sequence. The HHI encoding DNA was cloned into this Δ3-5-S35 containing vector using *Sac*II and *Bam*H_I restriction sites.

Example 27: Northern analysis

- RNA from the transduced MT2 cells were extracted and the presence of Δ3-5/ribozyme chimeric transcripts were assayed by Northern analysis (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). 30 Northern analysis of RNA extracted from MT2 transductants showed that Δ3-5/ribozyme chimeras of appropriate sizes were expressed (Fig. 35.36). In addition, these results demonstrated the relative differences in accumulation among the different constructs (Figure 35.36). The pattern of

expression seen from the Δ3-5/HHI ribozyme chimera was similar to 12 other ribozymes cloned into the Δ3-5 vector (not shown). In MT-2 cell line, Δ3-5/HHI ribozyme chimeras accumulated, on average, to less than 100 copies per cell.

- 5 Addition of a stem-loop onto the 3' end of Δ3-5/HHI did not lead to increased Δ3-5 levels (S3 in Fig. 35,36). The S5 construct containing both 5' and 3' stem-loop structures also did not lead to increased ribozyme levels (Fig. 35,36).

- 10 Interestingly, the S35 construct expression in MT2 cells was about 100-fold more abundant relative to the original Δ3-5/HHI vector transcripts (Fig. 35,36). This may be due to increased stability of the S35 transcript.

Example 28: Cleavage activity

- To assay whether ribozymes transcribed in the transduced cells contained cleavage activity, total RNA extracted from the transduced MT2 T cells were incubated with a labeled substrate containing the HHI cleavage site (Figure 37). Ribozyme activity in all but the S35 constructs, was too low to detect. However, ribozyme activity was detectable in S35-transduced T cell RNA. Comparison of the activity observed in the S35-transduced MT2 RNA with that seen with MT2 RNA in which varying amounts of in vitro transcribed S5 ribozyme chimeras, indicated that between 1-3 nM of S35 ribozyme was present in S35-transduced MT2 RNA. This level of activity corresponds to an intracellular concentration of 5,000-15,000 ribozyme molecules per cell.

Example 29: Clonal variation

- 25 Variation in the ribozyme expression levels among cells making up the bulk population was determined by generating several clonal cell lines from the bulk S35 transduced CEM line (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY) and the ribozyme expression and activity levels in the individual clones were measured (Figure 38 and 39).
- 30 All the individual clones were found to express active ribozyme. The ribozyme activity detected from each clone correlated well with the relative amounts of ribozyme observed by Northern analysis. Steady state ribozyme levels among the clones ranged from approximately 1,000 molecules per cell in clone G to 11,000 molecules per cell in clone H (Fig.

38). The mean accumulation among the clones, calculated by averaging the ribozyme levels of the clones, exactly equaled the level measured in the parent bulk population. This suggests that the individual clones are representative of the variation present in the bulk population.

5 The fact that all 14 clones were found to express ribozyme indicate that the percentage of cells in the bulk population expressing ribozyme is also very high. In addition, the lowest level of expression in the clones was still more than 10-fold that seen in bulk cells transduced with the original Δ3-5 vector. Therefore, the S35 gene unit should be much more effective
10 in a gene therapy setting in which bulk cells are removed, transduced and then reintroduced back into a patient.

Example 30: Stability

Finally, the bulk S35-transduced line, resistant to G418, was propagated for a period of 3 months (in the absence of G418) to determine
15 if ribozyme expression was stable over extended periods of time. This situation mimicks that found in the clinic in which bulk cells are transduced and then reintroduced into the patient and allowed to propagate. There was a modest 30% reduction of ribozyme expression after 3 months. This difference probably arose from cells with varying amount of ribozyme
20 expression and exhibiting different growth rates in the culture becoming slightly more prevalent in the culture. However, ribozyme expression is apparently stable for at least this period of time.

Example 31: Design and construction of TRZ-tRNA Chimera

A transcription unit, termed TRZ, is designed that contains the S35 motif (Figure 52). A desired RNA (e.g. ribozyme) can be inserted into the indicated region of TRZ tRNA chimera. This construct might provide additional stability to the desired RNA. TRZ-A and TRZ-B are non-limiting examples of the TRZ-tRNA chimera.

Referring to Fig. 53-54, a hammerhead ribozyme targeted to site I
30 (HHITRZ-A; Fig. 53) and a hairpin ribozyme (HPITRZ-A; Fig. 54), also targeted to site I, is cloned individually into the indicated region of TRZ tRNA chimera. The resulting ribozyme transcripts retain full RNA cleavage activity (see for example Fig. 55). Applicant has shown that efficient

expression of these TRZ tRNA chimera can be achieved in mammalian cells.

Besides ribozymes, desired RNAs like antisense, therapeutic editing RNAs, decoys, can be readily inserted into the indicated region of TRZ-
5 tRNA chimera to achieve therapeutic levels of RNA expression in mammalian cells.

Sequences listed in Figures 40-47 and 50 - 54 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (mutations, insertions and deletions) of the above examples can be readily
10 generated using techniques known in the art, are within the scope of the present invention.

Example 32: Ribozyme expression in T cell lines

Ribozyme expression in T cell lines stably-transduced with either a retroviral-based or an Adeno-associated virus (AAV)-based ribozyme
15 expression vector (Figure 56). The human T cell lines MT2 and CEM were transduced with either retroviral or AAV vectors encoding a neomycin selectable marker and a ribozyme (S35/HHI) expressed from pol III met; tRNA-driven promoter. Cells stably-transduced with the vectors were selectively expanded medium containing the neomycin antibiotic derivative, G418 (0.7 mg/ml). Ribozyme expression in the stable cell lines was then analyzed by Northern analysis. The probe used to detect
20 ribozyme transcripts also cross-hybridized with human met; tRNA sequences. Referring to Figure 56, S35/HHI RNA accumulates to significant levels in MT2 and CEM cells when transduced with either the retrovirus or
25 the AAV vector.

These are meant to be non-limiting examples, those skilled in the art will recognize that other vectors such as adenovirus vector (Figure 57), plasmid DNA vector, alpha virus vectors and the other derivatives thereof, can be readily generated to deliver the desired RNA, using techniques
30 known in the art and are within the scope of this invention. Additionally, the transcription units can be expressed individually or in multiples using pol II and/or pol III promoters.

References cited herein, as well as Draper WO 93/23569, 94/02495, 94/06331, Sullenger WO 93/12657, Thompson WO 93/04573, and Sullivan

WO 94/04609, and 93/11253 describe methods for use of vectors described herein, and are incorporated by reference herein. In particular these vectors are useful for administration of antisense and decoy RNA molecules.

5 Example 33: Ligated Ribozymes are catalytically active

The ability of ribozymes generated by ligation methods, described in Draper et al., PCT WO 93/23569, to cleave target RNA was tested on either matched substrate RNA (Fig. 58) or long (622 nt) RNA (Fig. 59, 60 and 61).

- Matched substrate RNAs were chemically synthesized using solid-phase RNA synthesis chemistry (Scaringe et al., 1990 Nucleic Acids Res. 18, 5433-5441). Substrate RNA was 5' end-labeled using [γ -³²P] ATP and polynucleotide kinase (Curr. Protocols Mol. Biol., 1992, ed. Ausubel et al., Wiley & Sons, NY). Ribozyme reactions were carried out under ribozyme excess conditions (k_{cat}/K_M; Herschlag and Cech, 1990 Biochemistry 29, 10159-10171). Briefly, ribozyme and substrate RNA were denatured and renatured separately by heating to 90°C and snap cooling on ice for 10 min in a buffer containing 50 mM Tris. HCl pH 7.5 and 10 mM MgCl₂. Cleavage reaction was initiated by mixing the ribozyme with the substrate at 37°C. Aliquots of 5 μ l were taken at regular intervals of time and the reaction was stopped by mixing with equal volume of formamide gel loading buffer (Curr. Protocols Mol. Biol., 1992, ed. Ausubel et al., Wiley & Sons, NY). The samples were resolved on 20 % polyacrylamide-urea gel. Referring to Fig. 58, -ΔG refers to the free energy of binding calculated for base-paired interactions between the ribozyme and the substrate RNA (Turner and Sugimoto, 1988 Supra). RPI A is a HH ribozyme with 6/6 binding arms. This ribozyme was synthesized chemically either as a one piece ribozyme or was synthesized in two fragments followed by ligation to generate a one piece ribozyme. The k_{cat}/K_M values for the two ribozymes were comparable.
- 30 A template containing T7 RNA polymerase promoter upstream of 622 nt long target sequence, was PCR amplified from a DNA clone. The target RNA (containing HH ribozyme cleavage sites B, C and D) was transcribed from this PCR amplified template using T7 RNA polymerase. The transcript was internally labeled during transcription by including [α -³²P] CTP as one 35 of the four ribonucleotide triphosphates. The transcription mixture was

treated with DNase-1, following transcription at 37°C for 2 hours, to digest away the DNA template used in the transcription. RNA was precipitated with Isopropanol and the pellet was washed two times with 70% ethanol to get rid of salt and nucleotides used in the transcription reaction. RNA is
5 resuspended in DEPC-treated water and stored at 4°C. Ribozyme cleavage reactions were carried out under ribozyme excess (kcat/KM) conditions [Herschlag and Cech 1990 *supra*]. Briefly, 1000 nM ribozyme and 10 nM internally labeled target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris.HCl, pH 7.5 and 10 mM MgCl₂. The RNAs were renatured by cooling to 37°C for 10-20 min.
10 Cleavage reaction was initiated by mixing the ribozyme and target RNA at 37°C. Aliquots of 5 µl were taken at regular intervals of time and the reaction was quenched by adding equal volume of stop buffer. The samples were resolved on a sequencing gel.

15 Example 34: Hammerhead ribozymes with ≥ 2 base-paired stem II are catalytically active

To decrease the cost of chemical synthesis of RNA, applicant was interested in determining whether the length of stem II region of a typical hammerhead ribozyme (≥ 4 bp stem II) can be shortened without decreasing the catalytic efficiency of the HH ribozyme. The length of stem II was systematically shortened by one base-pair at a time. HH ribozymes with three and two base-paired stem II were chemically synthesized using solid-phase RNA phosphoramidite chemistry (Scaringe *et al.*, 1990 *supra*).

25 Matched and long substrate RNAs were synthesized and ribozyme assays were carried out as described in example 33. Referring to figures 62, 63 and 64, data shows that shortening stem II of a hammerhead ribozyme does not significantly alter the catalytic efficiency. It is applicant's opinion that hammerhead ribozymes with ≥ 2 base-paired stem II region are catalytically active.

30 Example 35: Synthesis of catalytically active hairpin ribozymes

RNA molecules were chemically synthesized having the nucleotide base sequence shown in Fig. 65 for both the 5' and 3' fragments. The 3' fragments are phosphorylated and ligated to the 5' fragment essentially as described in example 37. As is evident from the Figure 65, the 3' and 5' fragments can hybridize together at helix 4 and are covalently linked via

GAAA sequence. When this structure hybridizes to a substrate, a ribozyme-substrate complex structure is formed. While helix 4 is shown as 3 base pairs it may be formed with only 1 or 2 base pairs.

- 40 nM mixtures of ligated ribozymes were incubated with 1-5 nM 5'
- 5 end-labeled matched substrates (chemically synthesized by solid-phase synthesis using RNA phosphoramidite chemistry) for different times in 50 mM Tris/HCl pH 7.5, 10 mM MgCl₂ and shown to cleave the substrate efficiently (Fig.66).

- The target and the ribozyme sequences shown in Fig. 62 and 65 are
10 meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using other sequences and techniques generally known in the art.

V. Constructs of Hairpin Ribozymes

- There follows an improved trans-cleaving hairpin ribozyme in which a new helix (*i.e.*, a sequence able to form a double-stranded region with another single-stranded nucleic acid) is provided in the ribozyme to base-pair with a 5' region of a separate substrate nucleic acid. This helix is provided at the 3' end of the ribozyme after helix 3 as shown in Figure 3. In addition, at least two extra bases may be provided in helix 2 and a portion of the substrate corresponding to helix 2 may be either directly linked to the 5' portion able to hydrogen bond to the 3' end of the hairpin or may have a linker of atleast one base. By trans-cleaving is meant that the ribozyme is able to act in *trans* to cleave another RNA molecule which is not covalently linked to the ribozyme itself. Thus, the ribozyme is not able to act on itself
25 in an intramolecular cleavage reaction.

By "base-pair" is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example Hoogsteen type) of interactions.

- The increase in length of helix 2 of a hairpin ribozyme (with or without
30 helix 5) has several advantages. These include improved stability of the ribozyme-target complex *in vivo*. In addition, an increase in the recognition sequence of the hairpin ribozyme improves the specificity of the ribozyme. This also makes possible the targeting of potential hairpin

ribozyme sites that would otherwise be inaccessible due to neighboring secondary structure.

The increase in length of helix 2 of a hairpin ribozyme (with or without helix 5) enhances *trans*-ligation reaction catalyzed by the ribozyme. *Trans*-ligation reactions catalyzed by the regular hairpin ribozyme (4 bp helix 2) is very inefficient (Komatsu *et al.*, 1993 *Nucleic Acids Res.* 21, 185). This is attributed to weak base-pairing interactions between substrate RNAs and the ribozyme. By increasing the length of helix 2 (with or without helix 5) the rate of ligation (*in vitro* and *in vivo*) can be enhanced several fold.

Results of experiments suggest that the length of H2 can be 6 bp without significantly reducing the activity of the hairpin ribozyme. The H2 arm length variation does not appear to be sequence dependent. HP ribozymes with 6 bp H2 have been designed against five different target RNAs and all five ribozymes efficiently cleaved their cognate target RNA. Additionally, two of these ribozymes were able to successfully inhibit gene expression (e.g., TNF- α) in mammalian cells. Results of these experiments are shown below.

HP ribozymes with 7 and 8 bp H2 are also capable of cleaving target RNA in a sequence-specific manner, however, the rate of the cleavage reaction is lower than those catalyzed by HP ribozymes with 6 bp H2.

Example 36: 4 and 6 base pair H2

Referring to Figures 67-72, HP ribozymes were synthesized as described above and tested for activity. Surprisingly, those with 6 base pairs in H2 were still as active as those with 4 base pairs.

25 **VI. Chemical Modification**

Oligonucleotides with 5'-C-alkyl Group

The introduction of an alkyl group at the 5'-position of a nucleoside or nucleotide sugar introduces an additional center of chirality into the sugar moiety. Referring to Fig. 75, the general structures of 5'-C-alkylnucleotides belonging to the D-allose, 2, and L-talose, 3, sugar families are shown. The family names are derived from the known sugars D-allose and L-talose ($R_1 = CH_3$ in 2 and 3 in Figure 75). Useful specific D-allose and L-talose

nucleotide derivatives are shown in Figure 76, 29-32 and Figure 77, 58-61 respectively.

- This invention relates to the use of 5'-C-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of
- 5 RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 5'-C-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 5'-C-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or
- 10 single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.
- 15 Also within the invention are 5'-C-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of
- 20 the 5'-C-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of
- 25 less consequence. Thus, for example, if a 5'-C-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in
- 30 the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 5'-C-alkylnucleosides, that is a nucleotide base having at the 5'-position on the sugar molecule an alkyl moiety. In a related aspect, the invention also features 5'-C-alkylnucleotides, and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably

includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above. In preferred embodiments, the sugar of the nucleoside or nucleotide is in an optically pure form, as the talose or allose sugar.

- 5 Examples of various alkyl groups useful in this invention are shown in Figure 75, where each R₁ group is any alkyl. These examples are not limiting in the invention. Specifically, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More
10 preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one
15 carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy,
20 =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons,
25 more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH.

- Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an
30 aromatic group which has at least one ring having a conjugated π electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an
35 alkyl group (as described above) covalently joined to an aryl group (as described above. Carbocyclic aryl groups are groups wherein the ring

- atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, 5 and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.
- 10 In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 5'-C-alkylnucleotides; e.g. enzymatic nucleic acids having a 5'-C-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic 15 molecule with at least one nucleotide having at its 5'-position an alkyl group. In other related aspects, the invention features 5'-C-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.
- 20 The 5'-C-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.
- 25 In another aspect, the invention features a method for conversion of a protected allo sugar to a protected talo sugar. In the method, the protected allo sugar is contacted with triphenyl phosphine, diethylazodicarboxylate, and *p*-nitrobenzoic acid under inversion causing conditions to provide the protected talo sugar. While one example of such conditions is provided 30 below, those in the art will recognize other such conditions. Applicant has found that such conversion allows for ready synthesis of all types of nucleotide bases as exemplified in the figures.
- 35 While this invention is applicable to all oligonucleotides, applicant has found that the modified molecules of this invention are particularly useful for enzymatic RNA molecules. Thus, below is provided examples of such

molecules. Those in the art will recognize that equivalent procedures can be used to make other molecules without such enzymatic activity. Specifically, Figure 1 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 1, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 5'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Substitutions of any nucleotide with any of the modified nucleotides shown in Figure 75 are possible.

The following are non-limiting examples showing the synthesis of nucleic acids using 5'-C-alkyl-substituted phosphoramidites and the syntheses of the amidites.

Example 37: Synthesis of Hammerhead Ribozymes Containing 5'-C-Alkyl-nucleotides & Other Modified Nucleotides

The method of synthesis would follow the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 26-29 and 56-59). These 5'-C-alkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 38: Methyl-2,3-O-Isopropylidine-6-Deoxy- β -D-allofuranoside (4)

A suspension of L-rhamnose (100 g, 0.55 mol), CuSO₄ (120 g) and conc. H₂SO₄ (4.0 mL) in 1.0 L of dry acetone was mixed for 24 h at RT, then filtered. Conc. NH₄OH (5 mL) was added to the filtrate and the newly formed precipitate was filtered. The residue was concentrated *in vacuo*, coevaporated with pyridine (2 x 300 mL), dissolved in pyridine (500 mL) and cooled to 0 °C. A solution of *p*-toluenesulfonylchloride (107 g, 0.56

mmol) in dry DCE (500 mL) was added dropwise over 0.5 h. The reaction mixture was left for 16 h at RT. The reaction was quenched by adding ice-water (0.5 L) and, after mixing for 0.5 h, was extracted with chloroform (0.75 L). The organic layer was washed with H₂O (2 x 500 mL), 10% H₂SO₄ (2 x 5 300 mL), water (2 x 300 mL), sat. NaHCO₃ (2 x 300 mL), brine (2 x 300 mL), dried over MgSO₄ and evaporated to dryness. The residue (115 g) was dissolved in dry MeOH (1 L) and treated with NaOMe (23.2 g, 0.42 mmol) in MeOH. The reaction mixture was left for 16 h at 20 °C, neutralized with dry CO₂ and evaporated to dryness. The residue was suspended in 10 chloroform (750 mL), filtered, concentrated to 100 mL and purified by flash chromatography in CHCl₃ to yield 45 g (37%) of compound 4.

Example 39: Methyl-2,3-O-Isopropylidine-5-O-t-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (5).

To solution of methylfuranoside 4 (12.5 g 62.2 mmol) and AgNO₃ 15 (21.25 g, 125.0 mmol) in dry DMF (300 mL) t-butyldiphenylsilyl chloride (22.2 g, 81 mmol) was added dropwise under Ar over 0.5 h. The reaction mixture was stirred for 4 h at RT, diluted with CHCl₃ (200 mL), filtered and evaporated to dryness (below 40 °C using a high vacuum oil pump). The residue was dissolved in CH₂Cl₂ (300 mL) washed with sat. NaHCO₃ (2 x 20 50 mL), brine (2 x 50 mL), dried over MgSO₄ and evaporated to dryness. The residue was purified by flash chromatography in CH₂Cl₂ to yield 20.0 g (75%) of compound 5.

Example 40: Methyl-5-O-t-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (6).

Methylfuranoside 5 (13.5 g, 30.6 mmol) was dissolved in 25 CF₃COOH:dioxane:H₂O / 2:1:1 (v/v/v, 200 mL) and stirred at 24 °C for 45 m. The reaction mixture was cooled to -10 °C, neutralized with conc. NH₄OH (140 mL) and extracted with CH₂Cl₂ (500 mL). The organic layer was separated, washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL), 30 dried over MgSO₄ and evaporated to dryness. The product 6 was purified by flash chromatography using a 0-10% MeOH gradient in CH₂Cl₂. Yield 9.0 g (76%).

Example 41: Methyl-2,3-di-O-Benzoyl-5-O-t-Butyldiphenylsilyl-6-Deoxy- β -D-Allofuranoside (7).

Methylfuranoside 6 (7.0 g, 17.5 mmol) was coevaporated with pyridine (2 x 100 mL) and dissolved in pyridine (100 mL). Benzoyl chloride 5 (5.4 g, 38.5 mmol) was added and the reaction mixture was left at RT for 16 h. Dry EtOH (50 mL) was added and the reaction mixture was evaporated to dryness after 0.5 h. The residue was dissolved in CH₂Cl₂ (300 mL), washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL), dried over MgSO₄ and evaporated to dryness. The product was purified by flash 10 chromatography in CH₂Cl₂ to yield 9.5 g (89%) of compound 7.

Example 42: 1-O-Acetyl-2,3-di-O-benzoyl-5-O-t-Butyldiphenylsilyl-6-Deoxy- β -D-Allofuranose (8).

Dibenzoate 7 (4.7 g, 7.7 mmol) was dissolved in a mixture of AcOH (10.0 mL), Ac₂O (20.0 mL) and EtOAc (30 mL) and the reaction mixture was 15 cooled 0 °C. 98% H₂SO₄ (0.15 mL) was then added. The reaction mixture was kept at 0 °C for 16 h, and then poured into a cold 1:1 mixture of sat. NaHCO₃ and EtOAc (150 mL). After 0.5 h of vigorous stirring the organic phase was separated, washed with brine (2 x 75 mL), dried over MgSO₄, 20 evaporated to dryness and coevaporated with toluene (2 x 50 mL). The product was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 4.0 g (82% as a mixture of α and β isomers).

Example 43: 1-(2',3'-di-O-Benzoyl-5'-O-t-Butyldiphenylsilyl-6'-Deoxy- β -D-Allofuranosyl)uracil (9).

Uracil (1.44 g, 11.5 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT, evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (6.36 g, 10.0 mmol) in dry CH₃CN (100 mL), followed 25 by CF₃SO₃SiMe₃ (2.8 g, 12.6 mmol). The reaction mixture was kept at 24 °C for 16 h, concentrated to 1/3 of its original volume, diluted with 100 mL of CH₂Cl₂ and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL), dried over MgSO₄, and evaporated to dryness. The product 9 was purified 30 by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 5.7 g (80%).

Example 44: N⁴-Benzoyl-1-(2',3'-Di-O-Benzoyl-5'-O-t-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)Cytosine (10).

- N⁴-benzoylcytosine (1.84 g, 8.56 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (3.6 g, 5.6 mmol) in dry CH₃CN (100 mL), followed by CF₃SO₃SiMe₃ (4.76 g, 21.4 mmol). The reaction mixture was boiled under reflux for 5 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. Purification by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ yielded 1.8 g (55%) of compound 10.
- 15 Example 45: N⁶-Benzoyl-9-(2',3'-di-O-Benzoyl-5'-O-t-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine (11).

N⁶-benzoyladenine (2.86 g, 11.86 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (7 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (3.6 g, 5.6 mmol) in dry CH₃CN (100 mL) followed by CF₃SO₃SiMe₃ (6.59 g, 29.7 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. The product 11 was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 2.7 g (60%).

- 30 Example 46: N²-Isobutyryl-9-(2',3'-di-O-Benzoyl-5'-O-t-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)guanine (12).

N²-Isobutyrylguanine (1.47 g, 11.2 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (6 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a

- solution of acetates **8** (3.4 g, 5.3 mmol) in dry CH₃CN (100 mL) followed by CF₃SO₃SiMe₃ (6.22 g, 28.0 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL),
5 brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. The product **12** was purified by flash chromatography using a gradient of 0-2% MeOH in CH₂Cl₂. Yield: 2.1g (54%).

Example 47: N⁶-Benzoyl-9-(2',3'-di-O-benzoyl-6'-Deoxy-β-D-Allofuranosyl)adenine (15).

- 10 Nucleoside **11** (1.65 g, 2.0 mmol) was dissolved in THF (50 mL) and a 1 M solution of TBAF in THF (4 mL) was added. The reaction mixture was kept at RT for 4 h, evaporated to dryness and the product purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ to yield 1.0 g (85%) of compound **15**.
- 15 **Example 48: N⁶-Benzoyl-9-(2',3'-di-O-Benzoyl-5'-O-Dimethoxytrityl-6'-Deoxy-β-D-Allofuranosyl)-adenine (19).**

Nucleoside **15** (0.55 g, 0.92 mmol) was dissolved in dry CH₂Cl₂ (50 mL). AgNO₃ (0.34 g, 2.0 mmol), dimethoxytrityl chloride (0.68 g, 2.0 mmol) and sym-collidine (0.48 g) were added under Ar. The reaction mixture was
20 stirred for 2h, diluted with CH₂Cl₂ (100 mL), filtered, evaporated to dryness and coevaporated with toluene (2 x 50 mL). Purification by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ yielded 0.8 g (97%) of compound **19**.

25 **Example 49: N⁶-Benzoyl-9-(5'-O-Dimethoxytrityl-6'-Deoxy-β-D-Allofuranosyl)adenine (23).**

- Nucleoside **19** (1.8 g, 2 mmol) was dissolved in dioxane (50 mL), cooled to 0 °C and 2 M NaOH (50 mL) was added. The reaction mixture was kept at 0 °C for 45 m, neutralized with Dowex 50 (Pyr⁺ form), filtered and the resin was washed with MeOH (2 x 50 mL). The filtrate was then
30 evaporated to dryness. Purification by flash chromatography using a gradient of 0-10% MeOH in CH₂Cl₂ yielded 1.1 g (80%) of **23**.

Example 50: N⁶-Benzoyl-9-(5'-O-Dimethoxytrityl-2'-O-t-butyldimethylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine (27).

Nucleoside 23 (1.2 g, 1.8 mmol) was dissolved in dry THF (50 mL). Pyridine (0.50 g, 8 mmol) and AgNO₃ (0.4 g, 2.3 mmol) were added. After 5 the AgNO₃ dissolved (1.5 h), t-butyldimethylsilyl chloride (0.35 g, 2.3 mmol) was added and the reaction mixture was stirred at RT for 16 h. The reaction mixture was diluted with CH₂Cl₂ (100 mL), filtered into sat. NaHCO₃ (50 mL), extracted, the organic layer washed with brine (2 x 50 mL), dried over MgSO₄ and evaporated to dryness. The product 27 was 10 purified by flash chromatography using a hexanes:EtOAc / 7:3 gradient. Yield: 0.7 g (50%).

Example 51: N⁶-Benzoyl-9-(5'-O-Dimethoxytrityl-2'-O-t-butyldimethylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine-3'-(2-Cyanoethyl N,N-diisopropyl-phosphoramidite) (31).

15 Standard phosphorylation of 27 according to Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidite 31 in 73% yield.

Example 52: Methyl-5-O-p-Nitrobenzoyl-2,3-O-Isopropylidine-6-deoxy-β-L-Talofuranoside (5)

20 Methylfuranoside 4 (3.1 g 14.2 mmol) was dissolved in dry dioxane (200 mL), p-nitrobenzoic acid (10.0 g, 60 mmol) and triphenylphosphine (15.74 g, 60.0 mmol) were added followed by DEAD (10.45 g, 60.0 mmol). The reaction mixture was left at RT for 16 h, EtOH (5 mL) was added, and after 0.5 h the reaction mixture was evaporated to dryness. The residue 25 was dissolved in CH₂Cl₂ (300 mL) washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL) dried over MgSO₄ and evaporated to dryness. Purification by flash chromatography using a hexanes:EtOAc / 9:1 gradient yielded 4.1 g (78%) of compound 33. Subsequent debenzoylation (NaOMe/MeOH) and silylation (see preparation of 5) led to L- 30 talofuranoside 34 which was converted to phosphoramidites 58-61 using the same methodology as described above for the preparation of the phosphoramidites of the D-allo-isomers 29-32.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage

or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan et al., PCT WO 94/ 02595.

5 The ribozymes and the target RNA containing site O were synthesized, deprotected and purified as described above. RNA cleavage assay was carried our at 37°C in the presence of 10 mM MgCl₂ as described above.

Applicant has substituted 5'-C-Me-L-talo nucleotides at positions A6,
10 A9, A9 + G10, C11.1 and C11.1 + G10, as shown in Figure 78 (HH-O1 to HH-05). *HH-O 1,2,4 and 5* showed almost wild type activity (Figure 79). However, *HH-03* demonstrated low catalytic activity. Ribozymes *HH-01, 2, 3, 4 and 5* are also extremely resistant to degradation by human serum nucleases.

15 Oligonucleotides with 2'-Deoxy-2'-Alkylnucleotide

This invention uses 2'-deoxy-2'-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 2'-deoxy-2'-alkylnucleotide-containing enzymatic
20 nucleic acids are catalytic nucleic molecules that contain 2'-deoxy-2'-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in
25 a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 2'-deoxy-2'-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides.
30 Contrary to the findings of De Mesmaeker et al. applicant has found that such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 2'-alkyl group may reduce binding affinity of the oligonucleotide
35 containing this modification, if that moiety is not in an essential base pair

forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 2'-deoxy-2'-alkyl-containing 5 molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which 10 are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 2'-deoxy-2'-alkylnucleotides, that is a nucleotide base having at the 2'-position on the sugar molecule an alkyl moiety and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the 15 invention preferably includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

Examples of various alkyl groups useful in this invention are shown in Figure 81, where each R group is any alkyl. The term "alkyl" does not 20 include alkoxy groups which have an "-O-alkyl" group, where "alkyl" is defined as described above, where the O is adjacent the 2'-position of the sugar molecule.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-deoxy-2'-alkylnucleotides 25 (preferably not a 2'-alkyl- uridine or thymidine); e.g. enzymatic nucleic acids having a 2'-deoxy-2'-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group. In other 30 related aspects, the invention features 2'-deoxy-2'-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-alkyl derivatives of this invention provide enhanced stability to 35 the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall

activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

- In another aspect, the invention features hammerhead motifs having enzymatic activity having ribonucleotides at locations shown in Figure 80 at 5, 6, 8, 12, and 15.1, and having substituted ribonucleotides at other positions in the core and in the substrate binding arms if desired. (The term "core" refers to positions between bases 3 and 14 in Figure 80, and the binding arms correspond to the bases from the 3'-end to base 15.1, and from the 5'-end to base 2). Applicant has found that use of ribonucleotides at these five locations in the core provide a molecule having sufficient enzymatic activity even when modified nucleotides are present at other sites in the motif. Other such combinations of useful ribonucleotides can be determined as described by Usman *et al. supra*.
- Figure 80 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 80 the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 2'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Although substitutions of any nucleotide with any of the modified nucleotides shown in Figure 81 are possible, and were indeed synthesized, the basic structure composed of primarily 2'-O-Me nucleotides with selected substitutions was chosen to maintain maximal catalytic activity (Yang *et al. Biochemistry* 1992, 31, 5005-5009 and Paoletta *et al. EMBO J.* 1992, 11, 1913-1919) and ease of synthesis, but is not limiting to this invention.
- Ribozymes from Figure 80 and Table 45 were synthesized and assayed for catalytic activity and nuclease resistance. With the exception of entries 8 and 17, all of the modified ribozymes retained at least 1/10 of the wild-type catalytic activity. From Table 45, all 2'-modified ribozymes showed very large and significant increases in stability in human serum (shown) and in the other fluids described below (Example 55, data not shown). The order of most aggressive nuclease activity was fetal bovine

serum, > human serum > human plasma > human synovial fluid. As an overall measure of the effect of these 2'-substitutions on stability and activity, a ratio β was calculated (Table 45). This β value indicated that all modified ribozymes tested had significant, >100 - >1700 fold, increases in 5 overall stability and activity. These increases in β indicate that the lifetime of these modified ribozymes *in vivo* are significantly increased which should lead to a more pronounced biological effect.

More general substitutions of the 2'-modified nucleotides from Figure 81 also increased the $t_{1/2}$ of the resulting modified ribozymes. 10 However the catalytic activity of these ribozymes was decreased > 10-fold.

In Figure 86 compound 37 may be used as a general intermediate to prepare derivatized 2'C-alkyl phosphoramidites, where X is CH₃, or an alkyl, or other group described above.

15 The following are non-limiting examples showing the synthesis of nucleic acids using 2'-C-alkyl substituted phosphoramidites, the syntheses of the amidites, their testing for enzymatic activity and nuclease resistance.

Example 53: Synthesis of Hammerhead Ribozymes Containing 2'-Deoxy-2'-Alkynucleotides & Other 2'-Modified Nucleotides

20 The method of synthesis used generally follows the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling 25 groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 10, 12, 17, 22, 31, 18, 26, 32, 36 and 38). Other 2'-modified phosphoramidites were prepared according to: 3 & 4, Eckstein *et al.* *International Publication No.* WO 92/07065; and 5 Kois *et al.* *Nucleosides & Nucleotides* 1993, 12, 1093-1109. The average stepwise 30 coupling yields were ~98%. The 2'-substituted phosphoramidites were incorporated into hammerhead ribozymes as shown in Figure 80. However, these 2'-alkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense

oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 54: Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 mM, 200 nM, 40 nM or 8 nM and the final substrate RNA concentrations were ~ 1 nM. Total reaction volumes were 50 mL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were initiated by mixing substrate and ribozyme solutions at t = 0. Aliquots of 5 mL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each time point was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis. Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

15 Example 55: Stability Assay

500 pmol of gel-purified 5'-end-labeled ribozymes were precipitated in ethanol and pelleted by centrifugation. Each pellet was resuspended in 20 mL of appropriate fluid (human serum, human plasma, human synovial fluid or fetal bovine serum) by vortexing for 20 s at room temperature. The samples were placed into a 37 °C incubator and 2 mL aliquots were withdrawn after incubation for 0, 15, 30, 45, 60, 120, 240 and 480 m. Aliquots were added to 20 mL of a solution containing 95% formamide and 0.5X TBE (50 mM Tris, 50 mM borate, 1 mM EDTA) to quench further nuclease activity and the samples were frozen until loading onto gels. Ribozymes were size-fractionated by electrophoresis in 20% acrylamide/8M urea gels. The amount of intact ribozyme at each time point was quantified by scanning the bands with a phosphorimager (Molecular Dynamics) and the half-life of each ribozyme in the fluids was determined by plotting the percent intact ribozyme vs the time of incubation and extrapolation from the graph.

Example 56: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-O-Phenoxythio-carbonyl-Uridine (7)

To a stirred solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-uridine, 6, (15.1 g, 31 mmol, synthesized according to *Nucleic Acid*

Chemistry, ed. Leroy Townsend, 1986 pp. 229-231) and dimethylamino-pyridine (7.57 g, 62 mmol) a solution of phenylchlorothionoformate (5.15 mL, 37.2 mmol) in 50 mL of acetonitrile was added dropwise and the reaction stirred for 8 h. TLC (EtOAc:hexanes / 1:1) showed disappearance
5 of the starting material. The reaction mixture was evaporated, the residue dissolved in chloroform, washed with water and brine; the organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel with EtOAc:hexanes / 2:1 as eluent to give 16.44 g (85%) of 7.

10 **Example 57: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl -Uridine (8)**

To a refluxing, under argon, solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-O-phenoxythiocarbonyl-uridine, 7, (5 g, 8.03 mmol) and allyltributyltin (12.3 mL, 40.15 mmol) in dry toluene, benzoyl peroxide
15 (0.5 g) was added portionwise during 1 h. The resulting mixture was allowed to reflux under argon for an additional 7-8 h. The reaction was then evaporated and the product 8 purified by flash chromatography on silica gel with EtOAc:hexanes / 1:3 as eluent. Yield 2.82 g (68.7%).

Example 58: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine (9)

20 A solution of 8 (1.25 g, 2.45 mmol) in 10 mL of dry tetrahydrofuran (THF) was treated with a 1 M solution of tetrabutylammoniumfluoride in THF (3.7 mL) for 10 m at room temperature. The resulting mixture was evaporated, the residue was loaded onto a silica gel column, washed with 1 L of chloroform, and the desired deprotected compound was eluted with
25 chloroform:methanol / 9:1. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched
30 with methanol (20 mL), evaporated, dissolved in chloroform, washed with 5% aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel, EtOAc:hexanes / 1:1 as eluent, to give 0.85 g (57%) of 9 as a white foam.

Example 59: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (10)

5'-O-Dimethoxytrityl-2'-C-allyl-uridine (0.64 g, 1.12 mmol) was dissolved in dry dichloromethane under dry argon. *N,N*-Diisopropylethylamine (0.39 mL, 2.24 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.35 mL, 1.57 mmol) was added dropwise to the stirred reaction solution and stirring was continued for 2 h at RT. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture 10 was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using a gradient of 10-60% EtOAc in hexanes containing 1% triethylamine mixture as eluent. Yield: 0.78 g (90%), white foam.

Example 60: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl-N⁴-Acetyl-Cytidine (11)

15 Triethylamine (6.35 mL, 45.55 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (5.66 g, 81.99 mmol) and phosphorous oxychloride (0.86 mL, 9.11 mmol) in 50 mL of anhydrous acetonitrile. To the resulting suspension a solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-C-allyl uridine (2.32 g, 4.55 mmol) in 30 mL of acetonitrile was 20 added dropwise and the reaction mixture was stirred for 4 h at room temperature. The reaction was concentrated *in vacuo* to a minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The 25 resulting foam was dissolved in 50 mL of 1,4-dioxane and treated with 29% aq. NH₄OH overnight at room temperature. TLC (chloroform:methanol / 9:1) showed complete conversion of the starting material. The solution was evaporated, dried by coevaporation with anhydrous pyridine and acetylated with acetic anhydride (0.52 mL, 5.46 mmol) in pyridine 30 overnight. The reaction mixture was quenched with methanol, evaporated, the residue was dissolved in chloroform, washed with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, evaporated to dryness and purified by flash chromatography on silica gel (3% MeOH in chloroform). Yield 2.3 g (90%) as a white foam.

Example 61: 5'-O-Dimethoxytrityl-2'-C-Allyl-N⁴-Acetyl-Cytidine

This compound was obtained analogously to the uridine derivative 9 in 55% yield.

Example 62: 5'-O-Dimethoxytrityl-2'-C-allyl-N⁴-Acetyl-Cytidine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (12)

2'-O-Dimethoxytrityl-2'-C-allyl-N⁴-acetyl cytidine (0.8 g, 1.31 mmol) was dissolved in dry dichloromethane under argon. N,N-Diisopropylethylamine (0.46 mL, 2.62 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.38 mL, 1.7 mmol) was added dropwise to a stirred reaction solution and stirring was continued for 2 h at room temperature. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using chloroform:ethanol / 98:2 with 2% triethylamine mixture as eluent. Yield: 0.91 g (85%), white foam.

Example 63: 2'-Deoxy-2'-Methylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine 14 (Hansske,F.; Madej,D.; Robins,M. J. *Tetrahedron* 1984, 40, 125 and Matsuda,A.; Takenuki,K.; Tanaka,S.; Sasaki,T.; Ueda,T. *J. Med. Chem.* 1991, 34, 812) (2.2 g, 4.55 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-uridine (1.0 g, 3.3 mmol, 72.5%) was eluted with 20% MeOH in CH₂Cl₂.

Example 64: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine (15)

2'-Deoxy-2'-methylene-uridine (0.91 g, 3.79 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes as eluant to yield 15 (0.43 g, 0.79 mmol, 22%).

Example 65: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (17)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl- β -D-ribofuranosyl)-uracil (0.43 g, 0.8 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.28 mL, 1.6 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.25 mL, 1.12 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.3 g, 0.4 mmol, 50%) was purified by flash column chromatography over silica gel using a 25-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.42 (CH₂Cl₂: MeOH / 15:1)

Example 66: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-Uridine

2'-Keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)uridine **14** (1.92 g, 12.6 mmol) and triphenylphosphine (2.5 g, 9.25 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol, 70%) eluted with 25% hexanes in EtOAc.

Example 67: 2'-Deoxy-2'-Difluoromethylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol, 68%) was eluted with 20% MeOH in CH₂Cl₂.

Example 68: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine (16)

2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.42 g, 4.18 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture

was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 5'-O-DMT-2'-deoxy-2'-difluoromethylene-uridine **16** (1.05 g, 1.8 mmol, 45%).

Example 69: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (18)

- 10 1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.577 g, 1 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.36 mL, 2 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.44 mL, 1.4 mmol). The reaction mixture
15 was stirred for 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.404 g, 0.52 mmol, 52%) was purified by flash chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂: MeOH / 15:1).
- 20 **Example 70: 2'-Deoxy-2'-Methylene-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-4-N-Acetyl-Cytidine 20**

Triethylamine (4.8 mL, 34 mmol) was added to a solution of POCl₃ (0.65 mL, 6.8 mmol) and 1,2,4-triazole (2.1 g, 30.6 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl) uridine **19** (1.65 g, 3.4 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at room temperature for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved
25 in dioxane (10 mL) and aq. ammonia (20 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (3 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5 mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The
30 residue was dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 2'-Deoxy-2'-Methylene-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-4-N-Acetyl-Cytidine **20** (0.85 g, 1.6 mmol, 48%).

35

organic extracts were dried over Na_2SO_4 , concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylsiloxane-1,3-diyl)-4-N-acetyl-cytidine **20** (1.3 g, 2.5 mmol, 73%) was eluted with 20% EtOAc in hexanes.

5 Example 71: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl- β -D-ribofuranosyl)-4-N-Acetyl-Cytosine **21**

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylsiloxane-1,3-diyl)-4-N-acetyl-cytidine **20** (1.3 g, 2.5 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The 10 residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol, 80%) was eluted with 10% MeOH in CH_2Cl_2 . 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.81 g, 2.4 mmol) in pyridine (10 mL) was added 15 dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. NaHCO_3 (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO_4 , concentrated *in vacuo* and 20 purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield **21** (0.88 g, 1.5 mmol, 75%).

Example 72: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl- β -D-ribofuranosyl)-4-N-Acetyl-Cytosine 3'-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite) (**22**)

25 1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl- β -D-ribofuranosyl)-4-N-acetyl-cytosine **21** (0.88 g, 1.5 mmol) dissolved in dry CH_2Cl_2 (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.8 mL, 4.5 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction 30 mixture was stirred 2 h at room temperature and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product **22** (0.82 g, 1.04 mmol, 69%) was purified by flash chromatography over silica gel using 50-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.36 (CH_2Cl_2 :MeOH / 20:1).

Example 73: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyl disiloxane-1,3-diyl)-4-N-Acetyl-Cytidine (24)

Et₃N (6.9 mL, 50 mmol) was added to a solution of POCl₃ (0.94 mL, 10 mmol) and 1,2,4-triazole (3.1 g, 45 mmol) in acetonitrile (20 mL) at 0 °C.

5 A solution of 2'-deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyl disiloxane-1,3-diyl)uridine 23 ([described in example 14] 2.6 g, 5 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at RT for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The

10 organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved in dioxane (20 mL) and aq. ammonia (30 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (5 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat.

15 NaHCO₃ (5mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyl disiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9

20 mmol, 78%) was eluted with 20% EtOAc in hexanes.

Example 74: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine (25)

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyl disiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol, 72%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol) was dissolved in

30 pyridine (10 mL) and a solution of DMT-Cl (1.03 g, 3.1 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO₄, concentrated *in*

35

vacuo and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield **25** (1.2 g, 1.9 mmol, 68%).

Example 75: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetylcytosine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (26)

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N-acetylcytosine **25** (0.6 g, 0.97 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.5 mL, 2.9 mmol) was added, followed by the dropwise addition of 2-10 cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product **26**, a white foam (0.52 g, 0.63 mmol, 65%) was purified by flash chromatography over silica gel using 30-70% EtOAc gradient in hexanes, 15 containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂:MeOH / 20:1).

Example 76: 2'-Keto-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-Butylbenzoyl)-Adenosine (28)

Acetic anhydride (4.6 mL) was added to a solution of 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine (Brown,J.; Christodolou, C.; Jones,S.; Modak,A.; Reese,C.; Sibanda,S.; Ubasawa A. *J. Chem .Soc. Perkin Trans. I* 1989, 1735) (6.2 g, 9.2 mmol) in DMSO (37 mL) and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with water. The organic layer was dried over MgSO₄ and 25 concentrated *in vacuo*. The residue was purified on a silica gel column to yield 2'-keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine **28** (4.8 g, 7.2 mmol, 78%).

Example 77: 2'-Deoxy-2'-methylene-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-Butylbenzoyl)-Adenosine (29)

30 Under a pressure of argon, sec-butyllithium in hexanes (11.2 mL, 14.6 mmol) was added to a suspension of triphenylmethylphosphonium iodide (7.07 g, 17.5 mmol) in THF (25 mL) cooled at -78 °C. The homogeneous orange solution was allowed to warm to -30 °C and a solution of 2'-keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine

- 28 (4.87 g, 7.3 mmol) in THF (25 mL) was transferred to this mixture under argon pressure. After warming to RT, stirring was continued for 24 h. THF was evaporated and replaced by CH₂Cl₂ (250 mL), water was added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl.
- 5 The organic layer was washed with H₂O (20 mL), 5% aqueous NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give the crude compound, which was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine 29 (3.86 g, 5.8 mmol, 79%).
- 10

Example 78: 2'-Deoxy-2'-Methylene-6-N-(4-t-Butylbenzoyl)-Adenosine

- 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine (3.86 g, 5.8 mmol) dissolved in THF (30 mL)
- 15 was treated with 1 M TBAF in THF (15 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-6-N-(4-t-butylbenzoyl)-adenosine (1.8 g, 4.3 mmol, 74%) was eluted with 10% MeOH in CH₂Cl₂.

- 20 Example 79: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-t-Butylbenzoyl)-Adenosine (29)

- 2'-Deoxy-2'-methylene-6-N-(4-t-butylbenzoyl)-adenosine (0.75 g, 1.77 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.66 g, 1.98 mmol) in pyridine (10 mL) was added dropwise over 15 m.
- 25 The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as an eluant to yield 29 (0.81 g, 1.1 mmol, 62%).
- 30

Example 80: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-t-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (31)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-6-N-(4-t-butylbenzoyl)-adenine 29 dissolved in dry CH₂Cl₂ (15 mL) was placed

in a round bottom flask under Ar. Diisopropylethylamine was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite. The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was 5 evaporated to a syrup *in vacuo* (40 °C). The product was purified by flash chromatography over silica gel using 30-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant (0.7 g, 0.76 mmol, 68%). R_f 0.45 (CH₂Cl₂: MeOH / 20:1)

Example 81: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-Butylbenzoyl)-Adenosine

2'-Keto-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine 28 (6.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium 15 chlorodifluoroacetate (2.3 g, 15 mmol) in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N- 20 (4-t-butylbenzoyl)-adenosine (4.1g, 6.4 mmol, 64%) eluted with 15% hexanes in EtOAc.

Example 82: 2'-Deoxy-2'-Difluoromethylene-6-N-(4-t-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-6-N-(4-t-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol, 77%) was eluted 30 with 20% MeOH in CH₂Cl₂.

Example 83: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-t-Butylbenzoyl)-Adenosine (30)

2'-Deoxy-2'-difluoromethylene-6-N-(4-t-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in

pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The 5 organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as eluant to yield 30 (2.6 g, 3.41 mmol, 69%).

Example 84: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-t-Butylbenzoyl)-Adenosine 3'-[2-Cyanoethyl N,N-diisopropylphosphoramidite])
10 **(32)**

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofurano-syl)-6-N-(4-t-butylbenzoyl)-adenine 30 (2.6 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (25 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the 15 dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.06 mL, 4.76 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). 32 (2.3 g, 2.4 mmol, 70%) was purified by flash column chromatography over silica gel using 20-50% EtOAc gradient in 20 hexanes, containing 1% triethylamine, as eluant. R_f 0.52 (CH₂Cl₂: MeOH / 15:1).

Example 85: 2'-Deoxy-2'-Methoxycarbonylmethylidine-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine (33)

Methyl(triphenylphosphoranylidine)acetate (5.4 g, 16 mmol) was 25 added to a solution of 2'-keto-3',5'-O-(tetraisopropyl disiloxane-1,3-diyl)-uridine 14 in CH₂Cl₂ under argon. The mixture was left to stir at RT for 30 h. CH₂Cl₂ (100 mL) and water were added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H₂O (20 mL), 5% aq. NaHCO₃ (20 mL), H₂O to neutrality, and 30 brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give crude product, that was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methoxycarbonylmethylidine-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine 33 (5.8 g, 10.8 mmol, 67.5%).

Example 86: 2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine (34)

Et₃N•3 HF (3 mL) was added to a solution of 2'-deoxy-2'-methoxycarbonylmethylidine-3',5'-O-(tetraisopropylsiloxyane-1,3-diyl)-uridine 33 (5 g, 9.3 mmol) dissolved in CH₂Cl₂ (20 mL) and Et₃N (15 mL). The resulting mixture was evaporated *in vacuo* after 1 h and chromatographed on a silica gel column eluting 2'-deoxy-2'-methoxycarbonylmethylidine-uridine 34 (2.4 g, 8 mmol, 86%) with THF:CH₂Cl₂ / 4:1.

Example 87: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine (35)

10 2'-Deoxy-2'-methoxycarbonylmethylidine-uridine 34 (1.2 g, 4.02 mmol) was dissolved in pyridine (20 mL). A solution of DMT-Cl (1.5 g, 4.42 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 2-5% MeOH in CH₂Cl₂ as an eluant to yield 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidine-uridine 35 (2.03 g, 3.46 mmol, 86%).

20 Example 88: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidine-Uridine 3'-{(2-cyanoethyl-N,N-diisopropylphosphoramidite)} (36)

1-(2'-Deoxy-2'-2'-methoxycarbonylmethylidine-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uridine 35 (2.0 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.91 mL, 4.08 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidine-uridine 3'-{(2-cyanoethyl-N,N-diisopropylphosphoramidite)} 36 (1.8 g, 2.3 mmol, 67%) was purified by flash column chromatography over silica gel using a 30-60% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.44 (CH₂Cl₂:MeOH / 9.5:0.5).

Example 89: 2'-Deoxy-2'-Carboxymethylidine-3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-Uridine 37

2'-Deoxy-2'-methoxycarbonylmethylidine-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine 33 (5.0 g, 10.8 mmol) was dissolved in MeOH (50 mL) and 1 N NaOH solution (50 mL) was added to the stirred solution at RT. The mixture was stirred for 2 h and MeOH removed *in vacuo*. The pH of the aqueous layer was adjusted to 4.5 with 1N HCl solution, extracted with EtOAc (2 x 100 mL), washed with brine, dried over MgSO₄ and concentrated *in vacuo* to yield the crude acid. 2'-Deoxy-2'-carboxymethylidine-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-uridine 37 (4.2 g, 7.8 mmol, 73%) was purified on a silica gel column using a gradient of 10-15% MeOH in CH₂Cl₂.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan *et al.* PCT WO 94/02595.

Oligonucleotides with 3' and/or 5' Dihalophosphonate

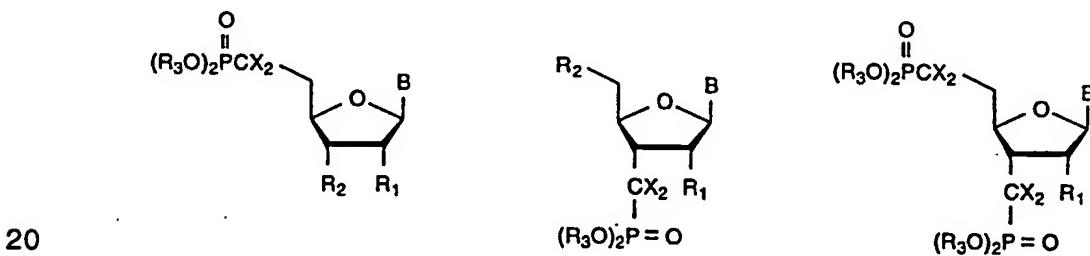
This invention synthesis and uses 3' and/or 5' dihalophosphonate-, e.g., 3' or 5'-CF₂-phosphonate-, substituted nucleotides that maintain or enhance the catalytic activity and/or nuclease resistance of an enzymatic or antisense molecule.

As the term is used in this application, 5'- and/or 3'-dihalophosphonate nucleotide containing ribozymes, deoxyribozymes (see Usman *et al.*, PCT/US94/11649, incorporated by reference herein), and chimeras of nucleotides, are catalytic nucleic molecules that contain 5'- and/or 3'-dihalophosphonate nucleotide components replacing, but not limited to, double-stranded stems, single-stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA or DNA transcript. This invention concerns

nucleic acids formed of standard nucleotides or modified nucleotides, which also contain at least one 5'-dihalophosphonate and/or one 3'-dihalophosphonate group.

- The synthesis of 1-O-Ac-2,3-di-O-Bz-D-ribofuranose 5-dihalomethylphosphonate in three steps from 1-O-methyl-2,3-O-isopropylidene- β -D-ribofuranose 5-deoxy-5-dihalomethylphosphonate is described (e.g., for the difluoro, in Figure 87). Condensation of this suitably derivatized sugar with silylated pyrimidines and purines affords novel nucleoside 5'-deoxy-5'-dihalomethylphosphonates. These intermediates may be incorporated into catalytic or antisense nucleic acids by either chemical (conversion of the nucleoside 5'-deoxy-5'-dihalomethylphosphonates into suitably protected phosphoramidites 12a or solid supports 12b, e.g., Figure 88) or enzymatic means (conversion of the nucleoside 5'-deoxy-5'-dihalomethylphosphonates into their triphosphates, e.g., 14 Figure 89, for T7 transcription).

Thus, in one aspect the invention features 5' and/or 3'-dihalonucleotides and nucleic acids containing such 5' and/or 3'-dihalonucleotides. The general structure of such molecules is shown below.



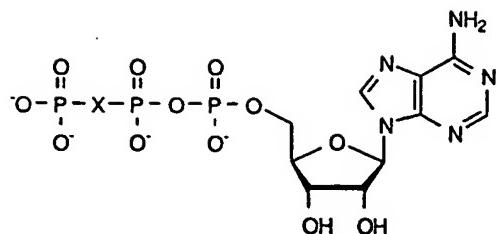
where R_1 is H, OH, or R, where R is a hydroxyl protecting group, e.g., acyl, alkysilyl, or carbonate; each R_2 is separately H, OH, or R; each R_3 is separately a phosphate protecting group, e.g., methyl, ethyl, cyanoethyl, p-nitrophenyl, or chlorophenyl; each X is separately any halogen; and each B is any nucleotide base.

The invention in particular features nucleic acid molecules having such modified nucleotides and enzymatic activity. In a related aspect the invention features a method for synthesis of such nucleoside 5'-deoxy-5'-dihalo and/or 3'-deoxy-3'-dihalophosphonates by condensing a

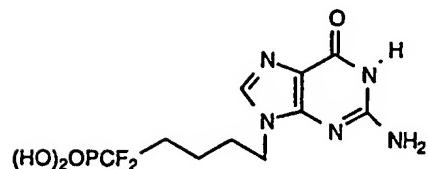
dihalophosphonate-containing sugar with a pyrimidine or a purine under conditions suitable to form a nucleoside 5'-deoxy-5'-dihalophosphonate and/or a 3'-deoxy-3'-dihalophosphonate.

Phosphonic acids may exhibit important biological properties
5 because of their similarity to phosphates (Engel, *Chem. Rev.* 1977, 77,
349-367). Blackburn and Kent (*J. Chem. Soc., Perkin Trans.* 1986, 913-
917) indicate that based on electronic and steric considerations α -fluoro
and α,α -difluoromethylphosphonates might mimic phosphate esters better
than the corresponding phosphonates. Analogues of pyro- and
10 triphosphates 1, where the bridging oxygen atoms are replaced by a
difluoromethylene group, have been employed as substrates in enzymatic
processes (Blackburn *et al.*, *Nucleosides & Nucleotides* 1985, 4, 165-167;
Blackburn *et al.*, *Chem. Scr.* 1986, 26, 21-24). 9-(5,5-Difluoro-5-
15 phosphonopentyl)guanine (2) has been utilized as a multisubstrate
analogue inhibitor of purine nucleoside phosphorylase (Halazy *et al.*, *J.
Am. Chem. Soc.* 1991, 113, 315-317). Oligonucleotides containing
methylene groups in place of phosphodiester 5'-oxygens are resistant
toward nucleases that cleave phosphodiester linkages between
phosphorus and the 5'-oxygen (Breaker *et al.*, *Biochemistry* 1993, 32,
20 9125-9128), but can still form stable complexes with complementary
sequences. Heinemann *et al.* (*Nucleic Acids Res.* 1991, 19, 427-433)
found that a single 3'-methylenephosphonate linkage had a minor
influence on the conformation of a DNA octamer double helix.

142



1



2



3

One common synthetic approach to α,α -difluoro-alkylphosphonates features the displacement of a leaving group from a suitable reactive substrate by diethyl (lithiodifluoromethyl)phosphonate (3) (Obayashi *et al.*,

- 5 *Tetrahedron Lett.* 1982, 23, 2323-2326). However, our attempts to synthesize nucleoside 5'-deoxy-5'-difluoro-methylphosphonates from 5'-deoxy-5'-iodonucleosides using 3 were unsuccessful, *i.e.* starting compounds were quantitatively recovered. The reaction of nucleoside 5'-aldehydes with 3, according to the procedure of Martin *et al.* (Martin *et al.*, 10 *Tetrahedron Lett.* 1992, 33, 1839-1842), led to a complex mixture of products. Recently, the synthesis of sugar α,α -difluoroalkylphosphonates from primary sugar triflates using 3 was described (Berkowitz *et al.*, *J. Org. Chem.* 1993, 58, 6174-6176). Unfortunately, our experience is that nucleoside 5'-triflates are too unstable to be used in these syntheses.

- 15 The following are non-limiting examples showing the synthesis of nucleoside 5'-deoxy-5'-difluoromethyl-phosphonates. Those in the art will recognize that equivalent methods can be readily devised based upon

these examples. These examples demonstrate that it is possible to achieve synthesis of 5'-deoxy-5'-difluoro derivatives in good yield and thus guide those in the art to such equivalent methods. The examples also indicate utility of such synthesis to provide useful oligonucleotides as described above.

Those in the art will recognize that useful modified enzymatic nucleic acids can now be designed, much as described by Draper et al., PCT/US94/13129 hereby incorporated by reference herein (including drawings).

10 **Example 90: Synthesis of Nucleoside 5'-Deoxy-5'-difluoromethylphosphonates**

Referring to Fig. 87, we synthesized a suitable glycosylating agent from the known D-ribose α,α -difluoromethylphosphonate (4) (Martin et al., *Tetrahedron Lett.* 1992, 33, 1839-1842) which served as a key
15 intermediate for the synthesis of nucleoside 5'-difluoromethylphosphonates.

Methyl 2,3-O-isopropylidene- β -D-ribofuranose α,α -difluoromethylphosphonate (4) was synthesized from the 5-aldehyde according to the procedure of Martin et al. (*Tetrahedron Lett.* 1992, 33, 20 1839-1842) (Figure 87). Removal of the isopropylidene group was accomplished under mild conditions (I_2 -MeOH, reflux, 18 h (Szarek et al., *Tetrahedron Lett.* 1986, 27, 3827) or Dowex 50 WX8 (H^+), MeOH, RT (about 20-25°C), 3 days) in 72% yield. The anomeric mixture thus obtained was benzoylated with benzoyl chloride/pyridine to afford the 2,3-
25 di-O-benzoyl derivative, which was subjected to mild acetolysis conditions (Walczak et al., *Synthesis*, 1993, 790-792) (Ac_2O , AcOH, H_2SO_4 , EtOAc, 0°C. The desired 1-O-acetyl-2,3-di-O-benzoyl-D-ribofuranose difluoromethylphosphonate (5) was obtained in quantitative yield as an anomeric mixture. These derivatives were used for selective glycosylation
30 of silylated uracil and N⁴-acetylcytosine under Vorbrüggen conditions (Vorbrüggen, *Nucleoside Analogs. Chemistry, Biology and Medical Applications*, NATO ASI Series A, 26, Plenum Press, New York, London, 1980; pp. 35-69. The use of $F_3CSO_2OSi(CH_3)_3$ as a glycosylation catalyst is precluded because it is expected to lead to the undesired 1-
35 ethyluracil or 9-ethyladenine byproducts: Podyukova, et al., *Tetrahedron*

Lett. 1987, 28, 3623-3626 and references cited therein) (SnCl_4 as a catalyst, boiling acetonitrile) to yield β -nucleosides (62% 6a, 75% 6b). Glycosylation of silylated N^6 -benzoyladenine under the same conditions yielded a mixture of N-9 isomer 6c and N-7 isomer 7 in 34% and 15%
5 yield, respectively. The above nucleotides were successfully deprotected using trimethylsilyl bromide for the cleavage of the ethyl groups, followed by treatment with ammonia-methanol to remove the acyl protecting groups. Nucleoside 5'-deoxy-5'-difluoromethylphosphonates 8 were finally purified on a DEAE Sephadex A-25 (HCO_3^-) column using a 0.01-0.25 M
10 TEAB gradient for elution and obtained as their sodium salts (82% 8a; 87% 8b; 82% 8c).

Selected analytical data: ^{31}P -NMR (^{31}P) and ^1H -NMR (^1H) were recorded on a Varian Gemini 400. Chemical shifts in ppm refer to H_3PO_4 and TMS, respectively. Solvent was CDCl_3 unless otherwise noted. 5: ^1H
15 δ 8.07-7.28 (m, Bz), 6.66 (d, $J_{1,2}$ 4.5, $\alpha\text{H}1$), 6.42 (s, $\beta\text{H}1$), 5.74 (d, $J_{2,3}$ 4.9, $\beta\text{H}2$), 5.67 (dd, $J_{3,2}$ 4.9, $J_{3,4}$ 6.6, $\beta\text{H}3$), 5.63 (dd, $J_{3,2}$ 6.7, $J_{3,4}$ 3.6, $\alpha\text{H}3$), 5.57 (dd, $J_{2,1}$ 4.5, $J_{2,3}$ 6.7, $\alpha\text{H}2$), 4.91 (m, H4), 4.30 (m, CH_2CH_3), 2.64 (m, CH_2CF_2), 2.18 (s, βAc), 2.12 (s, αAc), 1.39 (m, CH_2CH_3). ^{31}P δ 7.82 (t, $J_{\text{P},\text{F}}$ 105.2), 7.67 (t, $J_{\text{P},\text{F}}$ 106.5). 6a: ^1H δ 9.11 (s, 1H, NH), 8.01 (m, 11H, Bz, H6), 5.94 (d, $J_{1',2'}$ 4.1, 1H, H1'), 5.83 (dd, $J_{5,6}$ 8.1, 1H, H5), 5.79 (dd, $J_{2',1'}$ 4.1, $J_{2',3'}$ 6.5, 1H, H2'), 5.71 (dd, $J_{3',2'}$ 6.5, $J_{3',4'}$ 6.4, 1H, H3'), 4.79 (dd, $J_{4',3'}$ 6.4, $J_{4',\text{F}}$ 11.6, 1H, H4'), 4.31 (m, 4H, CH_2CH_3), 2.75 (tq, $J_{\text{H},\text{F}}$ 19.6, 2H, CH_2CF_2), 1.40 (m, 6H, CH_2CH_3). ^{31}P δ 7.77 (t, $J_{\text{P},\text{F}}$ 104.0). 8c:
20 ^{31}P (vs DSS) (D_2O) δ 5.71 (t, $J_{\text{P},\text{F}}$ 87.9).

25 Compound 7 was deacylated with methanolic ammonia yielding the product that showed λ_{max} (H_2O) 271 nm and λ_{min} 233 nm, confirming that the site of glycosylation was N-7.

Example 91: Synthesis of Nucleic Acids Containing Modified Nucleotide Containing Cores

30 The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.*, *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe *et al.*, *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end
35 (Figure 88 and Janda *et al.*, *Science* 1989, 244:437-440.). These

nucleoside 5'-deoxy-5'-difluoromethylphosphonates may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 introns, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

5 Example 92: Synthesis of Modified Triphosphate

The triphosphate derivatives of the above nucleotides can be formed as shown in Fig. 89, according to known procedures. *Nucleic Acid Chem.*, Leroy B. Townsend, John Wiley & Sons, New York 1991, pp. 337-340; *Nucleotide Analogs*, Karl Heinz Scheit; John Wiley & Sons New York 1980, 10 pp. 211-218.

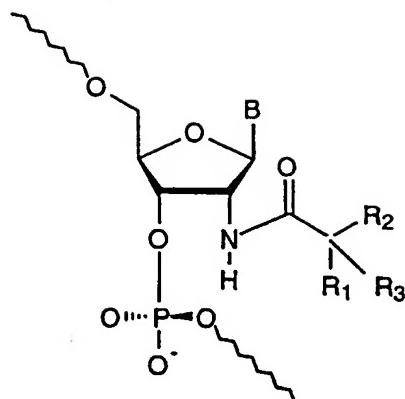
Equivalent synthetic schemes for 3' dihalophosphonates are shown in Figures 90 and 91 using art recognized nomenclature. The conditions can be optimized by standard procedures.

15 The nucleoside dihalophosphonates described herein are advantageous as modified nucleotides in any nucleic acid structure, e.g., catalytic or antisense, since they are resistant to exo- and endonucleases that normally degrade unmodified nucleic acids *in vivo*. They also do not perturb the normal structure of the nucleic acid in which they are incorporated thereby maintaining any activity associated with that structure.
20 These compounds may also be of use as monomers as antiviral and/or antitumor drugs.

Oligonucleotides with Amido or Peptido Modification

This invention replaces 2'-hydroxyl group of a ribonucleotide moiety with a 2'-amido or 2'-peptido moiety. In other embodiments, the 3' and 5' 25 portions of the sugar of a nucleotide may be substituted, or the phosphate group may be substituted with amido or peptido moieties. Generally, such a nucleotide has the general structure shown in Formula I below:

146



The base (B) is any one of the standard bases or is a modified nucleotide base known to those in the art, or can be a hydrogen group. In
 5 addition, either R₁ or R₂ is H or an alkyl, alkene or alkyne group containing between 2 and 10 carbon atoms, or hydrogen, an amine (primary, secondary or tertiary, e.g., R₃NR₄ where each R₃ and R₄ independently is hydrogen or an alkyl, alkene or alkyne having between 2 and 10 carbon atoms, or is a residue of an amino acid, i.e., an amide), an alkyl group, or
 10 an amino acid (D or L forms) or peptide containing between 2 and 5 amino acids. The zigzag lines represent hydrogen, or a bond to another base or other chemical moiety known in the art. Preferably, one of R₁, R₂ and R₃ is an H, and the other is an amino acid or peptide.

Applicant has recognized that RNA can assume a much more complex structural form than DNA because of the presence of the 2'-hydroxyl group in RNA. This group is able to provide additional hydrogen bonding with other hydrogen donors, acceptors and metal ions within the RNA molecule. Applicant now provides molecules which have a modified amine group at the 2' position, such that significantly more complex structures can be formed by the modified oligonucleotide. Such modification with a 2'-amido or peptido group leads to expansion and enrichment of the side-chain hydrogen bonding network. The amide and peptide moieties are responsible for complex structural formation of the oligonucleotide and can form strong complexes with other bases, and
 15 20 25 interfere with standard base pairing interactions. Such interference will allow the formation of a complex nucleic acid and protein conglomerate.

Oligonucleotides of this invention are significantly more stable than existing oligonucleotides and can potentially form biologically active bioconjugates not previously possible for oligonucleotides. They may also be used for *in vitro* selection of unique aptamers, that is, randomly 5 generated oligonucleotides which can be folded into an effective ligand for a target protein, nucleic acid or polysaccharide.

Thus, in one aspect, the invention features an oligonucleotide containing the modified base shown in Formula I, above.

In other aspects, the oligonucleotide may include a 3' or 5' nucleotide 10 having a 3' or 5' located amino acid or aminoacyl group. In all these aspects, as well as the 2'-modified nucleotide, it will be evident that various standard modifications can be made. For example, an "O" may be replaced with an S, the sugar may lack a base (i.e., abasic) and the phosphate moiety may be modified to include other substitutions (see 15 Sproat, *supra*).

Example 93: General procedure for the preparation of 2'-aminoacyl-2'-deoxy-2'-aminonucleoside conjugates.

Referring to Fig. 92, to the solution of 2'-deoxy-2'-amino nucleoside (1 mmol) and N-Fmoc L- (or D-) amino acid (1 mmol) in methanol 20 [dimethylformamide (DMF) and tetrahydrofuran (THF) can also be used], 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) [or 1-isobutyloxycarbonyl-2-isobutyloxy-1,2-dihydroquinoline (IIDQ)] (2 mmol) is added and the reaction mixture is stirred at room temperature or up to 50 °C from 3-48 hours. Solvents are removed under reduced pressure and 25 the residual syrup is chromatographed on the column of silica-gel using 1-10 % methanol in dichloromethane. Fractions containing the product are concentrated yielding a white foam with yields ranging from 85 to 95 %. Structures are confirmed by ¹H NMR spectra of conjugates which show correct chemical shifts for nucleoside and aminoacyl part of the molecule. 30 Further proofs of the structures are obtained by cleaving the aminoacyl protecting groups under appropriate conditions and assigning ¹H NMR resonances for the fully deprotected conjugate.

Partially protected conjugates described above are converted into their 5'-O-dimethoxytrityl derivatives and into 3'-phosphoramidites using 35 standard procedures (Oligonucleotide Synthesis: A Practical Approach,

M.J. Gait ed.; IRL Press, Oxford, 1984). Incorporation of these phosphoramidites into RNA was performed using standard protocols (Usman *et al.*, 1987 *supra*).

5 A general deprotection protocol for oligonucleotides of the present invention is described in Fig. 93.

The scheme shows synthesis of conjugate of 2'-d-2'-aminouridine. This is meant to be a non-limiting example, and those skilled in the art will recognize that, variations to the synthesis protocol can be readily generated to synthesize other nucleotides (e.g., adenosine, cytidine, 10 guanosine) and/or abasic moieties.

Example 94: RNA cleavage by hammerhead ribozymes containing 2'-aminoacyl modifications.

Hammerhead ribozymes targeted to site N (see Fig. 94) are synthesized using solid-phase synthesis, as described above. U4 and U7 15 positions are modified, individually or in combination, with either 2'-NH-alanine or 2'-NH-lysine.

RNA cleavage assay *in vitro*: Substrate RNA is 5' end-labeled using [γ -32P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace 20 amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction is initiated by mixing the 25 ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of 30 time.

Referring to Fig. 95, hammerhead ribozymes containing 2'-NH-alanine or 2'-NH-lysine modifications at U4 and U7 positions cleave the target RNA efficiently.

Sequences listed in Figure 94 and the modifications described in Figure 95 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 95: Aminoacetylation of 3'-ends of RNA

I. Referring to Fig. 96, 3'-OH group of the nucleotide is converted to succinate as described by Gait, *supra*. This can be linked with amino-alkyl solid support (for example: CpG). Zig-zag line indicates linkage of 3'OH group with the solid support.

II. Preparation of aminoacyl-derivatized solid support

A) Synthesis of O-Dimethoxytrityl (O-DMT) amino acids

15 Referring to Fig. 97, to a solution of L- (or D-) serine, tyrosine or threonine (2 mmol) in dry pyridine (15 ml) 4,4'-dimethoxytrityl chloride (3 mmol) is added and the reaction mixture is stirred at RT (about 20-25°C) for 16 h. Methanol (10 ml) is then added and the solution evaporated under reduced pressure. The residual syrup was partitioned between 5% aq.
20 NaHCO₃ and dichloromethane, organic layer was washed with brine, dried (Na₂SO₄) and concentrated *in vacuo*. The residue is purified by flash silicagel column chromatography using 2-10% methanol in dichloromethane (containing 0.5 % pyridine). Fractions containing product are combined and concentrated *in vacuo* to yield white foam (75-85 %
25 yield).

B) Preparation of the solid support and its derivatization with amino acids

Referring to Fig. 97, the modified solid support (has an OH group instead of the standard NH₂ end group) was prepared according to Haralambidis et al., Tetrahedron Lett. 1987, 28, 5199, (P denotes 30 aminopropyl CPG or polystyrene type support). O-DMT or NH-monomethoxytrityl (NH-MMT amino acid was attached to the above solid support using standard procedures for derivatization of the solid support (Gait, 1984, *supra*) creating a base-labile ester bond between amino acids

and the support. This support is suitable for the construction of RNA/DNA chain using suitably protected nucleoside phosphoramidites.

Example 96: Aminoacylation of 5'-ends of RNA

- I. Referring to Fig. 98, 5'-amino-containing sugar moiety was synthesized as described (Mag and Engels, 1989 *Nucleic Acids Res.* 17, 5973). Aminoacylation of the 5'-end of the monomer was achieved as described above and RNA phosphoramidite of the 5'-aminoacylated monomer was prepared as described by Usman *et al.*, 1987 *supra*. The phosphoramidite was then incorporated at the 5'-end of the oligonucleotide using standard solid-phase synthesis protocols described above.
- II. Referring to Fig. 99, aminoacyl group(s) is attached to the phosphate group at the 5'-end of the RNA using standard procedures described above.

VII. Reversing Genetic Mutations

- 15 Modification of existing nucleic acid sequences can be achieved by homologous recombination. In this process a transfected sequence recombines with homologous chromosomal sequences and can replace the endogenous cellular sequence. Boggs, 8 *International J. Cell Cloning* 80, 1990, describes targeted gene modification. It reviews the use of
- 20 homologous DNA recombination to correct genetic defects. Banga and Boyd, 89 *Proc. Natl. Acad. Sci. U.S.A.* 1735, 1992, describe a specific example of *in vivo* site-directed mutagenesis using a 50 base oligonucleotide. In this methodology a gene or gene segment is essentially replaced by the oligonucleotide used.
- 25 This invention uses a complementary oligonucleotide to position a nucleotide base changing activity at a particular site on a gene (RNA or genomic DNA), such that the nucleotide modifying activity will change (or revert) a mutation to wild-type, or its equivalent. By reversion or change of a mutation, we refer to reversion in a broad sense, such as when a
- 30 mutation at a second site which leads to functional reversion to a wild type phenotype. Also, due to the degeneracy of the genetic code, a revertant may be achieved by changing any one of the three codon positions. Additionally, creation of a stop codon in a deleterious gene (or transcript) is defined here as reverting a mutant phenotype to wild-type. An example of

this type of reversion is creating a stop codon in a critical HIV proviral gene in a human.

Referring to Figures 100 and 101, broadly there are two approaches to causing a site directed change in order to revert a mutation to wild-type.

- 5 In one (Fig. 100) the oligonucleotide is used to target RNA specifically. RNA is provided with a complementary (Watson-crick) oligonucleotide sequence to that in the target molecule. In this case the sequence modifying oligonucleotide would (analogously to an antisense oligonucleotide or ribozyme) have to be continuously present to revert the
- 10 RNA as it is made by the cell. Such a reversion would be transient and would potentially require continuous addition of more sequence modifying oligonucleotide. The transient nature of this approach is an advantage, in that treatment could be stopped by simply removing the sequence modifying oligonucleotide (as with a traditional drug).
- 15 A second approach targets DNA (Fig. 101) and has the advantage that changes may be permanently encoded in the target cell's genetic code. Thus, a single course (or several courses) of treatment may lead to permanent reversion of the genetic disease. If inadvertent chromosomal mutations are introduced this may cause cancer, mutate other genes, or
- 20 cause genetic changes in the germ-line (in patients of reproductive age). However, if the base changing activity is a specific methylation that may modulate gene expression it would not necessarily lead to germ-line transmission. See Lewin, Genes, 1983 John Wiley & Sons, Inc. NY pp 493-496.
- 25 Complementary base pairing to single-stranded DNA or RNA is one method of directing an oligonucleotide to a particular site of DNA. This could occur by a strand displacement mechanism or by targeting DNA when it is single-stranded (such as during replication, or transcription). Another method is using triple-strand binding (triplex formation) to double-stranded DNA, which is an established technique for binding poly-pyrimidine tracts, and can be extended to recognize all 4 nucleotides. See Povsic, T., Strobel, S., & Dervan, P. (1992). Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation. J. Am. Chem. Soc. **114**, 5934-5944 (1992). Knorre, D.G., Valentin, V.V.,
- 30 Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk,
- 35

1993) describe conjugation of reactive groups or enzyme to oligonucleotides and can be used in the methods described herein.

Recently, antisense oligonucleotides have been used to redirect an incorrect splice into order to obtain correct splicing of a splice mutant globin
5 gene *in vitro*. Dominski Z; Kole R (1993) Restoration of correct splicing in thalassemia pre-mRNA by antisense oligonucleotides. Proc Natl Acad Sci U S A 90:8673-7. Analogously, in one preferred embodiment of this invention a complementary oligomer is used to correct an existing mutant RNA, instead of the traditional approach of inhibiting that RNA by
10 antisense.

In either the RNA or DNA mode, after binding to a particular site on the RNA or DNA the oligonucleotide will modify the nucleic acid sequence. This can be accomplished by activating an endogenous enzyme (see Figure 102), by appropriate positioning of an enzyme (or ribozyme)
15 conjugated (or activated by the duplex) to the oligonucleotide, or by appropriate positioning of a chemical mutagen. Specific mutagens, such as nitrous acid which deaminates C to U, are most useful, but others can also be used if inactivation of a harmful RNA is desired.

RNA editing is a naturally occurring event in mammalian cells in
20 which a sequence modifying activity edits a RNA to its proper sequence post-transcriptionally. Higuchi, M., Single, F., Kohler, M., Sommer, B., and Seburg, P. (1993) RNA Editing of AMPA Receptor Subunit GluR-B: A base-paired intron-exon structure determines position and efficiency Cell 75:1361-1370. The machinery involved in RNA editing can be co-opted by
25 a suitable oligonucleotide in order to promote chemical modification.

The changes in the base created by the methods of this invention cause a change in the nucleotide sequence, either directly, or after DNA repair by normal cellular mechanisms. These changes functionally correct a genetic defect or introduce a stop codon. Thus, the invention is distinct
30 from techniques in which an active chemical group (e.g., an alkylator) is attached to an antisense or triple strand oligonucleotide in order to chemically inactivate the target RNA or DNA.

Thus, this invention creates an alteration to an existing base in a nucleic acid molecule so that the base is read *in vivo* as a different base.

This includes correcting a sequence instead of inactivating a gene but can also include inactivating a deleterious gene.

Thus, in one aspect, the invention features a method for altering *in vivo* the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule. The method includes contacting the nucleic acid molecule *in vivo* with an oligonucleotide or peptide nucleic acid or other sequence specific binding molecules able to form a duplex or triplex molecule with the nucleic acid molecule. After formation of the duplex or triplex molecule a base modifying activity chemically or enzymatically alters the targeted base directly, or after nucleic acid repair *in vivo*. This results in the functional alteration of the nucleic acid sequence.

By "alter", as it is used in this context, is meant that one or more chemical moieties in a targeted base, or bases, is altered so that the mutant nucleic acid will be functionally different. Thus, this is distinct from prior methods of correcting defects in DNA, such as homologous recombination, in which an entire segment of the targeted sequence is replaced with a segment of DNA from the transfected nucleic acid. This is also distinct from other methods that use reactive groups to inactivate a RNA or DNA target, in that this method functionally corrects the sequence of the target, instead of merely damaging it, by causing it to be read by a polymerase as a different base from the original base. As noted above, the naturally occurring enzymes in a cell can be utilized to cause the chemical alteration, examples of which are provided below.

By "functionally alter" is meant that the ability of the target nucleic acid to perform its normal function (*i.e.*, transcription or translation control) is changed. For example, an RNA molecule may be altered so that it can cause production of a desired protein, or a DNA molecule can be altered so that upon DNA repair, the DNA sequence is changed.

By "mutant" it is meant a nucleic acid molecule which is altered in some way compared to equivalent molecules present in a normal individual. Such mutants may be well known in the art, and include, molecules present in individuals with known genetic deficiencies, such as muscular dystrophy, or diabetes and the like. It also includes individuals with diseases or conditions characterized by abnormal expression of a gene, such as cancer, thalassemia's and sickle cell anemia, and cystic

fibrosis. It allows modulation of lipid metabolism to reduce artery disease, treatment of integrated AIDS genomes, and AIDS RNA, and Alzheimer's disease. Thus, this invention concerns alteration of a base in a mutant to provide a "wild type" phenotype and/or genotype. For deleterious conditions this involves altering a base to allow expression or prevent expression as is necessary. When treating an infection, such as HIV, it concerns inactivation of a gene in the HIV RNA by mutation of the mutant (*i.e.*, non-human gene) to a wild type (*i.e.*, no production of a non-human protein). Such modification is performed *in trans* rather than *in cis* as in prior methods.

In preferred embodiments, the oligonucleotide is of a length (at least 12 bases, preferably 17 - 22) sufficient to activate dsRNA deaminase *in vivo* to cause conversion of an adenine base to inosine; the oligonucleotide is an enzymatic nucleic acid molecule that is active to chemically modify a base (see below); the nucleic acid molecule is DNA or RNA; the oligonucleotide includes a chemical mutagen, *e.g.*, the mutagen is nitrous acid; and the oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methylation of cytosine to 5-methylcytosine.

In a most preferred embodiment, the invention features correction of a mutation, rather than inactivation of a target by causing a mutation.

Using *in vitro* directed evolution, it is possible to screen for ribozymes with catalytic activities different than RNA cleavage. Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. *Science* 261:1411-1418. Using these methods of *in vitro* directed evolution, an enzymatic nucleic acid molecule, or ribozyme that mutates bases, instead of cleaving the phosphodiester backbone can be selected. This is a convenient method of obtaining an enzyme with the appropriate base sequence modifying activities for use in the present invention.

Sequence modifying activities can change one nucleotide to another (or modify a nucleotide so that it will be repaired by the cellular machinery to another nucleotide). Sequence modifying activities could also delete or add one or more nucleotides to a sequence. A specific embodiment of adding sequences is described by Sullenger and Cech, PCT/US94/12976

hereby incorporated by reference herein), in which entire exons with wild-type sequence are spliced into a mutant transcript. The present invention features only the addition of a few bases (1 - 3).

Thus, in another aspect, the invention features ribozymes or 5 enzymatic nucleic acid molecules active to change the chemical structure of an existing base in a separate nucleic acid molecule. Applicant is the first to determine that such molecules would be useful, and to provide a description of how such molecules might be isolated.

Molecules used to achieve *in situ* reversion can be delivered using 10 the existing means employed for delivering antisense molecules and ribozymes, including liposomes and cationic lipid complexes. If the *in situ* reverting molecule is composed only of RNA, then expression vectors can be used in a gene therapy protocol to produce the reverting molecules endogenously, analogously to antisense or ribozymes expression vectors. 15 There are several advantages of using such an expression vector, rather than simply replacing the gene through standard gene therapy. Firstly, this approach would limit the production of the corrected gene to cells that already express that gene. Furthermore, the corrected gene would be properly regulated by its natural transcriptional promoter. Lastly, reversion 20 can be used when the mutant RNA creates a dominant gain of function protein (e.g., in sickle cell anemia), where correction of the mutant RNA is necessary to stop the production of the deleterious mutant protein, and allow production of the corrected protein.

Endogenous Mammalian RNA Editing System

25 It was observed in the mid-1980s that the sequence of certain cellular RNAs were different from the DNA sequence that encodes them. By a process called RNA editing, cellular RNA are post-transcriptionally modified to a) create a translation initiation and termination codons, b) enable tRNA and rRNA to fold into a functional conformation (for a review 30 see Bass, B. L. (1993) In The RNA World, R. Gesteland, R. and Atkins, J. eds. (Cold Spring Harbor, New York; CSH Lab. Press) pp. 383-418). The process of RNA editing includes base modification, deletion and insertion of nucleotides.

Although, the RNA editing process is widespread among lower 35 eukaryotes, very few RNAs (four) have been reported to undergo editing in

mammals (Bass, *supra*). The predominant mode of RNA editing in mammalian system is base modification (C → U and A → G). The mechanism of RNA editing in the mammalian system is postulated to be that C→U conversion is catalyzed by cytidine deaminase. The mechanism 5 of conversion of A→G has recently been reported for glutamate receptor B subunit (gluR-B) in rat PC12 cells (Higuchi, M. et al. (1993) *Cell* 75, 1361-1370). According to Higuchi gluR-B mRNA precursor attains a structure such that intron 11 and exon 11 can form a stable stem-loop structure. This stem-loop structure is a substrate for a nuclear double strand-specific 10 adenine deaminase enzyme. The deamination will result in the conversion of A→I. Reverse transcription followed by double strand synthesis will result in the incorporation of G in place of A.

In the present invention, the endogenous deaminase activity or other such activities can be utilized to achieve targeted base modification.

15 The following are examples of the invention to illustrate different methods by which *in vivo* conversion of a base can be achieved. These are provided only to clarify specific embodiments of the invention and are not limiting to the invention. Those in the art will recognize that equivalent methods can be readily devised within the scope of the claims.

20 Example 97: Exploiting cellular dsRNA dependent Adenine to Inosine converter:

An endogenous activity in most mammalian cells and *Xenopus* oocytes converts about 50% of adenines to inosines in double stranded RNA. (Bass, B. L., & Weintraub, H. (1988). An unwinding activity that 25 covalently modifies its double-stranded RNA substrate. *Cell*, 55, 1089-1098.). This activity can be used to cause an *in situ* reversion of a mutation at the RNA level. Referring to Figures 102 and 104, for demonstration purposes a stop codon is incorporated into the coding region of dystrophin, which is fused to the reporter gene luciferase. This 30 stop codon can be reverted by targeting an antisense RNA which is long enough to activate the dsRNA deaminase, which converts Adenines to Inosines. The A to I transition will be read by the ribosome as an A to G transition in some cases and will thereby functionally revert the stop codon. While other A's in this region may be converted to I's and read as G, 35 converting an A to I (G) cannot create a stop codon. The A to I transitions

in the region surrounding the target mutation will create some point mutations, however, the function of the dystrophin protein is rarely inactivated by point mutations.

The reverted mRNA was then translated in a cell lysate and assayed
5 for luciferase activity. As evidenced by the dramatic increase in luciferase counts in the graph in figure 103, the A to I transition was read by the ribosome as an A to G transition and the stop codon has successfully been reverted with the lysate treated complex. As a control, an irrelevant non-complementary RNA oligonucleotide was added to the
10 dystrophin/luciferase mRNA. As expected, in this case no translation (luciferase activity) is observed because of the stop codon. As an additional control, the hybrid was not treated with extract, and again no translation (luciferase activity) is observed (Figure 103).

While other A's in the targeted region may have been converted to I's
15 and read as G, converting an A to I (G) cannot create a stop codon, so the ribosome will still read through the region. Dystrophin is not generally sensitive to point mutations if the open reading frame is maintained, so a dystrophin protein made from an mRNA reverted by this method should retain full activity.

20 The following detail specifics of the methodology: RNA oligonucleotides were synthesized on a 394 (ABI) synthesizer using phosphoramidite chemistry. The sequence of the synthetic complementary RNA that binds to the mutant dystrophin sequence is as follows (5' to 3'):

CCCGCGGTAGATCTTCTGGAGGCTTACAGTTTCTACAAACCTCC
25 CTTCAAA (Seq. ID No. 1)

Referring to Figure 104, fifty-nine base pairs of a human dystrophin mutant sequence containing a stop codon was fused in frame to the luciferase coding region using standard cloning technology, into the *Hind* III and *Not I* sites of pRC-CMV (Invitrogen, San Diego, CA). The AUG of
30 luciferase was deleted. The sequences of the insert from the *Hind* III site to the start of the luciferase coding region is (5' to 3'):

GCCCCTGAGGAGCGATGGAGGCCTTGAAGGGAGGTTGTGGAAAA
CTGTAAGCCTCCAGAAAGATCTACCGCGG (Seq ID No. 2)

This corresponds to base pairs 3649-3708 of normal dystrophin (Entrez ID # 311627) with a Sac II site at the 3' end. This plasmid was used as a template for *in vitro* transcription of mRNA using T7 polymerase with the manufacturers protocol (Promega, Madison, WI).

- 5 *Xenopus* nuclear extracts were prepared in 0.5X TGKED buffer (0.5X= 25mM Tris (pH 7.9), 12.5% glycerol, 25 mM KCl, 0.25mM DTT and 0.05mM EDTA), by vortexing nuclei and resuspended in a volume of 0.5X TGKED equal to total cytoplasm volume of the oocytes. Bass, B.L. & Weintraub, H. *Cell* 55; 1089-1098 (1988).
- 10 The target mRNA at 500ng/ul was pre-annealed to 1 micromolar complementary or irrelevant RNA oligonucleotide by heating to 70°C, and allowing it to slowly cool to 37°C over 30 minutes. Fifty nanograms of mRNA pre-annealed to the RNA oligonucleotides was added to 7ul of nuclear extracts containing 1mM ATP, 15mM EDTA, 1600un/ml RNasin 15 and 12.5mM Tris pH 8 to a total volume of 12ul. Bass, B.L. & Weintraub, H. *supra*. This mixture, which contains the dsRNA deaminase activity, was incubated for 30 minutes at 25°C. Next, 1.5ul of this mixture was added to a rabbit reticulocyte lysate *in vitro* translation mixture and translated for two hours according to the manufacturers protocol (Life Technologies, 20 Gaithersberg, MD), except that an additional 1.3 mM magnesium acetate was added to compensate for the EDTA carried through from the nuclear extract mixture. Luciferase assays were performed on 15ul of extract with the Promega luciferase assay system (Promega, Madison, WI), and luminescence was detected with a 96 well luminometer, and the results are 25 displayed in the graph in figure 102.

Example 98: Base changing activities

- The chemical synthesis of antisense and triple-strand forming oligomers conjugated to reactive groups is well studied and characterized (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova, 30 O.S. *Design and targeted reactions of oligonucleotide derivatives 1-366* (CRC Press, Novosibirsk, 1993) and Povsic, T., Strobel, S. & Dervan, P. Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation *J. Am. Chem. Soc.* 114, 5934-5944 35 (1992). Reactive groups such as alkylators that can modify nucleotide bases in targeted RNA or DNA have been conjugated to oligonucleotides.

Additionally enzymes that modify nucleic acids have been conjugated to oligonucleotides. (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk, 1993). In the past these 5 conjugated chemical groups or enzymes have been used to inactivate DNA or RNA that is specifically targeted by antisense or triple-strand interactions. Below is a list of useful base changing activities that could be used to change the sequence of DNA or RNA targeted by antisense or triple strand interactions, in order to achieve *in situ* reversion of mutations, 10 as described herein (see figure 100-104).

1. Deamination of 5-methylcytosine to create thymidine (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993). Also, nitrous acid or related compounds promote oxidative deamination of 15 C to be read at T(Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.). Additionally hydroxylamine or related compounds can transform C to be read at T (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.)
- 20 2. Deamination of cytosine to create uracil (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993) or by chemical groups similar to nitrous acid that promote oxidative deamination (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.)
- 25 3. Deamination of Adenine to be read like G (Inosine) (as done by the adenosine deaminase, AMP deaminase or the dsRNA deaminating activity (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993).
- 30 4. Methylation of cytosine to 5-methylcytosine
5. Transforming thymidine (or uracil) to O²-methyl thymidine (or O²-methyl uracil), to be read as cytosine by alkynitrosoureas (Xu, and Swann, *Tetrahedron Letters* 35:303-306 (1994)).

6. Transforming guanine to 6-O-methyl (or other alkyls) to be
read as adenine (Mehta and Ludlum, Biochimica et Biophysica Acta,
521:770-778 (1978) which can be done with the mutagen ethyl methane
sulfonate (EMS) Microbial Genetics, David Freifelder, Jones and Bartlett
5 Publishers, Inc., Boston, 1987, PP.226-230.

7. Amination of uracil to cytosine (as performed by the cellular
enzyme CTP synthetase (Bass, B.L. in *The RNA World* (Cold Spring Harbor
Laboratory Press, Cold Spring Harbor, 1993).

The following are examples of useful chemical modifications that can
10 be utilized in the present invention. There are a few preferred
straightforward chemical modifications that can change one base to
another base. Appropriate mutagenic chemicals are placed on the
targetting oligonucleotide, e.g., nitrous acid, or a suitable protein with such
activity. Such chemicals and proteins can be attatched by standard
15 procedures. These include molecules which introduce fundamental
chemical changes, that would be useful independent of the particular
technical approach. See Lewin, Genes, 1983 John Wiley & Sons, Inc. NY
pp 42-48.

The following matrix shows that the chemical modifications noted can
20 cause transversion reversions (pyrimidine to pyrimidine, or purine to
purine) in RNA or DNA. The transversions (pyrimidine to purine, or purine
to pyrimidine) are not preferred because these are more difficult chemical
transformations. The footnotes refer to the specific desired chemical
transformations. The bold footnotes refer to the reaction on the opposite
25 DNA strand. For example, if one desires to change an A to a G, this can be
accomplished at the DNA level by using reaction #5 to change a T to a C in
the opposing strand. In this example an A/T base pair goes to A/C, then
when the DNA is replicated, or mismatch repair occurs this can become
G/C, thus the original A has been converted to a G.

30

ISR matrix**Reverted Base**

Mutant base	A	T(U)	C	G
-------------	---	------	---	---

A	-	Transversion	Transversion	DNA ^{5,3} /RNA ³
T(U)	Transversion	-	DNA ⁵ /RNA ⁷	Transversion
C	Transversion	RNA ² /DNA ⁶	-	Transversion
G	DNA ⁶ /RNA ⁶	Transversion	Transversion	-

- 1 Deamination of 5-methylcytosine to create thymidine.
- 2 Deamination of cytosine to create uracil.
- 3 Deamination of Adenine to be read like G (Inosine).
- 5 4 Methylation of cytosine to 5-methylcytosine.
- 5 5 Transforming thymidine (or uracil) to O²-methyl thymidine (or O²-methyl uracil), to be read as cytosine (Xu, and Swann, Tetrahedron Letters 35:303-306 (1994)).
- 10 6 Transforming guanine to 6-O-methyl (or other alkyls) to be read as adenine (Mehta and Ludlum, Biochimica et Biophysica Acta, 521:770-778 (1978)).
7. Amination of uracil to cytosine. Bass *supra*. fig. 6c.

In Vitro Selection Strategy

Referring to Figure 105, there is provided a schematic describing an approach to selecting for a ribozyme with such base changing activity. An RNA is designed that folds back on itself (this is similar to approaches already used to select for RNA ligases, Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. Science 261:1411-1418). A degenerate loop opposing the base to be modified provides for diversity. After incubating this library of molecules in a buffer, the RNA is reverse transcribed into DNA (that is, using standard *in vitro* evolution protocol. Tuerk and Gold, 249 Science 505, 1990) , and then the DNA is selected for having a base change. A restriction enzyme cleavage and size selection or its equivalent is used to isolate the fraction of DNAs with the appropriate base change. The cycle could then be repeated many times.

The *in vitro* selection (evolution) strategy is similar to approaches developed by Joyce (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641; Joyce, G. F. (1992) Scientific American 267, 90-97) and Szostak (Bartel, D. and Szostak, J. (1993) Science 261:1411-1418; Szostak, J. W. 5 (1993) TIBS 17, 89-93). Briefly, a random pool of nucleic acids is synthesized wherein, each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to 10 bind to its target (the region flanking the mutant nucleotide), 2) complimentary DNA (cDNA) synthesis and PCR amplification of molecules selected for their base modifying activity, 3) introduction of restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides 15 represented at every position within the random region) or the degeneracy can be partial (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences (see Figure 105), such that the degenerate domain is placed across from the mutant base (the base that is targeted for 20 modification). This random library of nucleic acids is incubated under conditions that ensure folding of the nucleic acids into conformations that facilitate the catalysis of base modification (the reaction protocol may also include certain cofactors like ATP or GTP or an S-adenosyl-methionine (if methylation is desired) in order to make the selection more stringent). 25 Following incubation, nucleic acids are converted into complimentary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with base modification (at the mutant base position) can be separated from rest of the population of nucleic acids by using a variety of methods. For example, a restriction endonuclease cleavage site can either be created or abolished 30 as a result of base modification. If a restriction endonuclease site is created as a result of base modification, then the library can be digested with the restriction endonuclease (RE). The fraction of the population that is cleaved by the RE is the population that has been able to catalyze the base modification reaction (active pool). A new piece of DNA (containing 35 oligonucleotide primer binding sites for PCR and RE sites for cloning) is ligated to the termini of the active pool to facilitate PCR amplification and subsequent cycles (if necessary) of selection. The final pool of nucleic acids with the best base modifying activity is cloned in to a plasmid vector

and transformed into bacterial hosts. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

5 Base modifying enzymatic nucleic acids (identified via in vitro selection) can be used to cause the chemical modification *in vivo*.

In addition, the ribozyme could be evolved to specifically bind a protein having an enzymatic base changing activity.

10 Such ribozymes can be used to cause the above chemical modifications *in vivo*. The ribozymes or above noted antisense-type molecules can be administered by methods discussed in the above referenced art.

VIII. Administration of Nucleic Acids

15 Applicant has determined that double-stranded nucleic acid lacking a transcription termination signal can be used for continuous expression of the encoded RNA. This is achieved by use of an R-loop, *i.e.*, an RNA molecule non-covalently associated with the double-stranded nucleic acid and which causes localized denaturation ("bubble" formation) within the double stranded nucleic acid (Thomas et al., 1976 Proc. Natl. Acad. Sci. USA 73, 2294). In addition, applicant has determined that the RNA portion of the R-loop can be used to target the whole R-loop complex to a desirable intracellular or cellular site, and aid in cellular uptake of the complex. Further, applicant indicates that expression of enzymatically active RNA or ribozymes can be significantly enhanced by use of such R-loop complexes.

25 Thus, in one aspect, the invention features a method for introduction of enzymatic nucleic acid into a cell or tissue. A complex of a first nucleic acid encoding the enzymatic nucleic acid and a second nucleic acid molecule is provided. The second nucleic acid molecule has sufficient complementarity with the first nucleic acid to be able to form an R-loop base pair structure under physiological conditions. The R-loop is formed in a region of the first nucleic acid molecule which promotes expression of RNA from the first nucleic acid under physiological conditions. The method further includes contacting the complex with a cell or tissue under

conditions in which the enzymatic nucleic acid is produced within the cell or tissue.

- By "complex" is simply meant that the two nucleic acid molecules interact by intermolecular bond formation (such as by hydrogen bonding) 5 between two complementary base-paired sequences. The complex will generally be stable under physiological condition such that it is able to cause initiation of transcription from the first nucleic acid molecule.

The first and second nucleic acid molecules may be formed from any desired nucleotide bases, either those naturally occurring (such as 10 adenine, guanine, thymine and cytosine), or other bases well known in the art, or may have modifications at the sugar or phosphate moieties to allow greater stability or greater complex formation to be achieved. In addition, such molecules may contain non-nucleotides in place of nucleotides. Such modifications are well known in the art, see e.g., Eckstein *et al.*, 15 International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science, 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Sproat,B. European Patent Application 20 92110298.4 which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.

By "sufficient complementarity" is meant that sufficient base pairing occurs so that the R-loop base pair structure can be formed under the 25 appropriate conditions to cause transcription of the enzymatic nucleic acid. Those in the art will recognize routine tests by which such sufficient base pairs can be determined. In general, between about 15 - 80 bases is sufficient in this invention.

By "physiological condition" is meant the condition in the cell or 30 tissue to be targeted by the first nucleic acid molecule, although the R-loop complex may be formed under many other conditions. One example is use of a standard physiological saline at 37°C, but it is simply desirable in this invention that the R-loop structure exists to some extent at the site of action so that the expression of the desired nucleic acid will be achieved at that 35 site of action. While it is preferred that the R-loop structure be stable under

- those conditions, even a minimal amount of formation of the R-loop structure to cause expression will be sufficient. Those in the art will recognize that measurement of such expression is readily achieved, especially in the absence of any promoter or leader sequence on the first
- 5 nucleic acid molecule (Daube and von Hippel, 1992 Science 258, 1320). Such expression can thus only be achieved if an R-loop structure is truly formed with the second nucleic acid. If a promoter or leader sequence is provided, then it is preferred that the R-loop be formed at a site distant from those regions so that transcription is enhanced.
- 10 In a related aspect, the invention features a method for introduction of ribonucleic acid within a cell or tissue by forming an R-loop base-paired structure (as described above) with the first nucleic acid molecule lacking any promoter region or transcription termination signal such that once expression is initiated it will continue until the first nucleic acid is degraded.
- 15 In another related aspect, the invention features a method in which the second nucleic acid is provided with a localization factor, such as a protein, e.g., an antibody, transferin, a nuclear localization peptide, or folate, or other such compounds well known in the art, which will aid in targeting the R-loop complex to a desired cell or tissue.
- 20 In preferred embodiments, the first nucleic acid is a plasmid, e.g., one without a promoter or a transcription termination signal; the second nucleic acid is of length between about 40-200 bases and is formed of ribonucleotides at a majority of positions; and the second nucleic acid is covalently bonded with a ligand such as a nucleic acid, protein, peptide,
- 25 lipid, carbohydrate, cellular receptor, nuclear localization factor, or is attached to maleimide or a thiol group; the first nucleic acid is an expression plasmid lacking a promoter able to express a desired gene, e.g., it is a double-stranded molecule formed with a majority of deoxyribonucleic acids; the R-loop complex is a RNA/DNA heteroduplex;
- 30 no promoter or leader region is provided in the first nucleic acid; and the R-loop is adapted to prevent nucleosome assembly and is designed to aid recruitment of cellular transcription machinery.

In other preferred embodiments, the first nucleic acid encodes one or more enzymatic nucleic acids, e.g., it is formed with a plurality of

intramolecular and intermolecular cleaving enzymatic nucleic acids to allow release of therapeutic enzymatic nucleic acid *in vivo*.

In a further related aspect, the invention features a complex of the above first nucleic acid molecules and second nucleic acid molecules.

5 R-loop complex

An R-loop complex is designed to provide a non-integrating plasmid so that, when an RNA polymerase binds to the plasmid, transcription is continuous until the plasmid is degraded. This is achieved by hybridizing an RNA molecule, 40 to 200 nucleotides in length, to a DNA expression 10 plasmid resulting in an R-loop structure (see figure 106). This RNA, when conjugated with a ligand that binds to a cell surface receptor, triggers internalization of the plasmid/RNA-ligand complex. Formation of R-loops in general is described by DeWet, 1987 Methods in Enzymol. 145, 235; Neuwald et al., 1977 J. Virol. 21,1019; and Meyer et al., 1986 J. Ult. Mol. 15 Str. Res. 96, 187. Thus, those in the art can readily design complexes of this invention following the teachings of the art.

Promoters placed in retroviral genomes have not always behaved as planned in that the additional promoter will serve as a stop signal or reverses the direction of the polymerase. Applicant was told that creation 20 of an R-loop between the promoter and the reporter gene increased the transfection efficiency. Incubation of an RNA molecule with a double-stranded DNA molecule, containing a region of complementarity with the RNA will result in the formation of a stable RNA-DNA heteroduplex and the DNA strand that has a sequence identical to the RNA will be displaced into 25 a loop-like structure called the R-loop. This displacement of DNA strand occurs because an RNA-DNA duplex is more stable compared to a DNA-DNA duplex. Applicant was also told that an 80 nt long RNA was used to generate a R-loop structure in a plasmid encoding the β -galactosidase gene. The R-loop was initiated either in the promoter region or in the 30 leader sequence. Plasmids containing an R-loop structure were microinjected into the cytoplasm of COS cells and the gene expression was assayed. R-loop formation in the promoter region of the plasmid inhibited expression of the gene. RNA that hybridized to the leader sequence between the promoter and the gene, or directly to the first 80 35 nucleotides of the mRNA increased the expression levels 8-10 fold. The

proposed mechanism is that R-loop formation prevents nucleosome assembly, thus making the DNA more accessible for transcription. Alternatively, the R-loop may resemble a RNA primer promoting either DNA replication or transcription (Daube and von Hippel, 1992, supra).

- 5 One of the salient features of this invention is to generate R-loops in expression vectors of choice and introduce them into cells to achieve enhanced expression from the expression vector. The presence of an R-loop may aid in the recruitment of cellular transcription machinery. Once an RNA polymerase binds to the plasmid and initiates transcription, the
10 process will continue until a termination signal is reached, or the plasmid is degraded.

This invention will increase the expression of ribozymes inside a cell. The idea is to construct a plasmid with no transcription termination signal, such that a transcript-containing multiple ribozyme units can be
15 generated. In order to liberate unit length ribozymes, self-processing ribozymes can be cloned downstream of each therapeutic ribozyme (see figure 107) as described by Draper *supra*.

Ligand Targeting

- Another salient feature of this invention is that the RNA used to generate R-loop structures can be covalently linked to a ligand (nucleic acid, proteins, peptides, lipids, carbohydrates, etc.). Specific ligands can be chosen such that the ligand can bind selectively to a desired cell surface receptor. This ligand-receptor interaction will help internalize a plasmid containing an R-loop. Thus, RNA is used to attach the ligand to the
20 DNA such that localization of the gene to certain regions of the cell is achieved. One of several methods can be used to attach a ligand to RNA. This includes the incorporation of deoxythymidine containing a 6 carbon spacer having a terminal primary amine into the RNA (see figure 108). This amino group can be directly derivatized with the ligand, such as folate (Lee
25 and Low, 1994 J. Biol. Chem. 269, 3198-3204). The RNA containing a 6 carbon spacer with a terminal amine group is mixed with folate and the mixture is reacted with activators like 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC). This reaction should be carried
30 out in the presence of 1-Hydroxybenzotriazole hydrate (HOBT) to prevent
35 any undesirable side reactions.

The RNA can also be derivatized with a heterobifunctional crosslinking agent (or linker) like succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB). The SMPB introduces a maleimide into the RNA. This maleimide can then react with a thiol moiety either in a peptide or in a protein. Thiols can also be introduced into proteins or peptides that lack naturally occurring thiols using succinylacetylthioacetate. The amino linker can be attached at the 5' end or 3' end of the RNA. The RNA can also contain a series of nucleotides that do not hybridize to the DNA and extend the linker away from the RNA/DNA complex, thus increasing the accessibility of the ligand for its receptor and not interfering with the hybridization. These techniques can be used to link peptides such as nuclear localization signal (NLS) peptides (Lanford et al., 1984 Cell 37, 801-813; Kalderon et al., 1984 Cell 39, 499-509; Goldfarb et al., 1986 Nature 322, 641-644) and/or proteins like the transferrin (Curiel et al., 1991 Proc. Natl. Acad. Sci. USA 88, 8850-8854; Wagner et al., 1992 Proc. Natl. Acad. Sci. USA 89, 6099-6103; Giulio et al., 1994 Cell. Signal. 6, 83-90) to the ends of R-loop forming RNA in order to facilitate the uptake and localization of the R-loop-DNA complex. To link a protein to the ends of R-loop forming RNA, an intrinsic thiol can be used to react with the maleimide or the thiols can be introduced into the protein itself using either iminothiolate or succinimidyl acetyl thioacetate (SATA; Duncan et al., 1983 Anal. Biochem 132, 68). The SATA requires an additional deprotection step using 0.5 M hydroxylamine.

In addition liposomes can be used to cause an R-loop complex to be delivered to an appropriate intracellular cite by techniques well known in the art. For example, pH-sensitive liposomes (Connor and Huang, 1986 Cancer Res. 46, 3431-3435) can be used to facilitate DNA transfection.

Calcium phosphate mediated or electroporation-mediated delivery of the R-loop complex in to desired cells can also be readily accomplished.

30 In vitro Selection

In vitro selection strategies can be used to select nucleic acids that a) can form stable R-loops b) selectively bind to specific cell surface receptors. These nucleic acids can then be covalently linked to each other. This will help internalize the R-loop-containing plasmid efficiently using receptor-mediated endocytosis. The *in vitro* selection (evolution) strategy is

similar to approaches developed by Joyce (Beaudry and Joyce, 1992 Science 257, 635-641; Joyce, 1992 Scientific American 267, 90-97) and Szostak (Bartel and Szostak, 1993 Science 261:1411-1418; Szostak, 1993 TIBS 17, 89-93). Briefly, a random pool of nucleic acids is
5 synthesized wherein each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (a specific region of the double strand DNA), 2)
10 complimentary DNA (cDNA) synthesis and PCR amplification of molecules selected for their affinity to form R-loop and/or their ability to bind to a specific receptor, 3) introduction of a restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every
15 position within the random region) or the degeneracy can be partial (Beaudry and Joyce, 1992 Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences. This random library of nucleic acids is incubated under conditions that ensure equilibrium binding to either double-stranded DNA or cell surface
20 receptor. Following incubation, nucleic acids are converted into complementary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with desired characteristics can be separated from the rest of the population of nucleic acids by using a variety of methods (Joyce, 1992 supra). The desired pool of nucleic acids can then be carried through
25 subsequent rounds of selection to enrich the population with the most desired traits. These molecules are then cloned in to appropriate vectors. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

30 Other embodiments are within the following claims.

TABLE ICharacteristics of Ribozymes**Group I Introns**

Size: ~200 to >1000 nucleotides.

Requires a U in the target sequence immediately 5' of the cleavage site.

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

RNaseP RNA (M1 RNA)

Size: ~290 to 400 nucleotides.

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group all are bacterial in origin.

Hammerhead Ribozyme

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site.

Binds a variable number nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figures 1 and 2).

Hairpin Ribozyme

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site.

Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme

Size: 50 - 60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

Neurospora VS RNA Ribozyme

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.
Sequence requirements not fully determined.
Binding sites and structural requirements not fully determined. Only 1
known member of this class. Found in *Neurospora* VS RNA (Figure 5).

Table 2
Human ICAM HH Target sequence

nt. Position	Target Sequences	nt. Position	Target Sequences
11	CCCCAGU C GACCGTUG	386	ACCGUGU A CUUGGACU
23	CUGAGCU C CUCUGCU	394	CUGGACU C CAGAACG
26	AGCUCUU C UGGCUACU	420	CACCCCU C CCTUCUU
31	CUCUGCU A CUCAGAG	425	CUCCCCU C UUGGCAG
34	UGCUCACU C AGAGUUG	427	CCCCUCU U GGCAGCC
40	UCAGAGU U GCAACCU	450	AGAACCU U ACCCUAC
48	GCAACCU C AGCCUCG	451	GAACCUU A CCCUACG
54	UCAGCCU C GCUAUGG	456	UUACCCU A CGCUGCC
58	CCUCGCU A UGGCUCC	495	CCAACCU C ACGUGGG
64	UAUGGCU C CCAGCAG	510	UGCUGCU C CGUGGGGG
96	CCGCACU C CUGGUCC	564	CUGAGGU C ACCGACCA
102	UCCUGGU C CUGCUCG	592	GAGAGAU C ACCAUGG
108	UCCUGCU C GGGGCUU	607	AGCCAAU U UCUCGUG
115	CGGGGCU C UGUUOCU	608	GCCAAUU U CUCGUGC
119	GCUCUGU U CCCAGGA	609	CCAATUU C UCGUGCC
120	CUCUGUU C CCAGGAC	611	AAUUUCU C GUCCCGC
146	CAGACAU C UGUGUCC	656	GAGCUGU U UGAGAAC
152	UCUGUGU C CCCCUCU	657	AGCUGUU U GAGAACAA
158	UCCCCCU C AAAAGUC	668	AACACCU C GGCCCCC
165	CAAAGU C AUCCUGC	677	GCCCCCU A CCAGCUC
168	AAGUCAU C CUGCCCC	684	ACCAGCU C CAGACCU
185	GGAGGCCU C CGUGCUG	692	CAGACCU U UGUCUUG
209	AGCACCU C CUGUGAC	693	AGACCUU U GUCCUGC
227	CCCAAGU U GUUGGGC	696	CCUUUGU C CUGCCAG
230	AAGUUGU U GGGCAUA	709	AGCGACU C CCCCACA
237	UGGGCAU A GAGACCC	720	CACAACU U GUCAGCC
248	ACCCCGU U GCCUAAA	723	AACIUUGU C AGCCCCC
253	GUUGCCU A AAAAGGA	735	CCCCGGU C CUAGAGG
263	AAGGAGU U GCUCCUG	738	GGGUCCU A GAGGUGG
267	AGUUGCU C CUGCCUG	765	CCGUGGU C UGUUCCC
293	AAGGUGU A UGAACUG	769	GGUCUGU U CCCUGGA
319	AGAAGAU A GCCAAC	770	GUCUGUU C CCUGGAC
335	AUGUGCU A UUCAAAC	785	GGGCUGU U CCCAGUC
337	GUGCUAL U CAAACUG	786	GGCUGUU C CCAGUCU
338	UGCUALU C AAACUGC	792	UCCCAGU C UCGGAGG
359	GGGCAGU C AACAGCU	794	CCAGUCU C GGAGGCC
367	AACAGCU A AAACCUU	807	CCCAGGU C CACCUUGG
374	AAAACCU U CCUCACC	833	CAGAGGU U GAACCCC
375	AAACCUU C CUCACCG	846	CCACAGU C ACCUAUG
378	CCUUCCU C ACCGUGU	851	GUACCU A UGGCAAC

863	AACGACU C CUUCUCG	1408	UCGACAU C UUGAGGG
866	GACUCCU U CUUGGCC	1410	GAGAUCU U GAGGGCA
867	ACUCCUU C UGGGCCA	1421	GGCACCU A CCUCUGU
869	UCCUUCU C GGCCAAG	1425	CCUACCU C UGUCCGGG
881	AAGGCCU C AGUCAGU	1429	CCUCUGU C GGGCCAG
885	CCUCAGU C AGUGUGA	1444	GAGCACU C AAGGGGA
933	GUGCAGU A AUACUGG	1455	GGGAGGU C ACCCGCG
936	CAGUAAU A CUGGGGA	1482	AUGUGGU C UCCCCCCC
978	UGACCAU C UACAGCU	1484	GUGCUCU C CCCCCGG
980	ACCAUCU A CAGCUUU	1493	CCCCGGU A UGAGAUU
986	UACAGCU U UCGGGCG	1500	AUGAGAU U GUCAUCA
987	ACAGCUU U CGGGCGC	1503	AGAUUGU C AUCAUCA
988	CAGCUUU C CGGGGCC	1506	UUGUCAU C AUCACUG
1005	ACGUGAU U CUGACGA	1509	UCAUCAU C ACUGUGG
1006	CGUGAUU C UGAOGAA	1518	CUGUGGU A GCAGCCG
1023	CAGAGGU C UCAGAAC	1530	CCGCAGU C AUAAUGG
1025	GAGGUCU C AGAAGGG	1533	CAGUCAU A AUGGGCA
1066	CCACCCU A GAGCCAA	1551	CAGGCCU C AGCACGU
1092	AUGGGGU U CCAGCCC	1559	AGCACGU A CCUCUAU
1093	UGGGGUU C CAGCCCA	1563	CGUACCU C UAUAACC
1125	CCCAGCU C CUGCUGA	1565	UACCUUCU A UAACCGC
1163	CGCAGCU U CUCCUGC	1567	CCUCUAU A ACGGCCA
1164	GCAGCUU C UCCUGCU	1584	GGAAGAU C AAGAAA
1166	AGCUUCU C CUGCUUC	1592	AAGAAA U CAGACUA
1172	UCCUGCU C UGCAACC	1599	ACAGACU A CAACAGG
1200	GCCAGCU U AUACACA	1651	CACGCCU C CCUGAAC
1201	CCAGCUU A UACACAA	1661	UGAACCU A UCCCGGG
1203	AGCUUUAU A CACAAGA	1663	AACCUAU C CGGGGAC
1227	GGGAGCU U CGUGUCC	1678	AGGCCU C UUCCUOG
1228	GGAGCUU C GUGUCCU	1680	GGCCUCU U CCUOGGC
1233	UUCGUGU C CUGUAUG	1681	GCCUCUU C CUOGGCC
1238	GUCCUGU A UGGCCCC	1684	UCUJCCU C GGCCUUC
1264	GAGGGAU U GUCCGGG	1690	UCGGCCU U CCCAUAU
1267	GGAUUGU C CGGGAAA	1691	CGGCCUU C CCAUAUU
1294	AGAAAAAU U CCCAGCA	1696	UCCCAU A UUGGUGG
1295	GAAAAAU C CCAGCAG	1698	CCCAUAU U GGUGGCA
1306	GCAGACU C CAAUGUG	1737	AAGACAU A UGCCAUG
1321	CCAGGCU U GGGGGAA	1750	UGCAGCU A CACCUAC
1334	AACCCAU U GCCCGAG	1756	UACACCU A CGGGCCC
1344	CCGAGCU C AAGUGUC	1787	AGGGCAU U GUCCUCA
1351	CAAGUGU C UAAAGGA	1790	GCAUUGU C CUCAGUC
1353	AGUGUCU A AAGGAUG	1793	UUGUCCU C AGUCAGA
1366	UGGCACU U UCCCACU	1797	CCUCAGU C AGAUACA
1367	GGCACUU U CCCACUG	1802	GUCAAGAU A CAAACAGC
1368	GCACUUU C CCACUGC	1812	ACAGCAU U UGGGGCC
1380	UGCCCCAU C GGGGAAU	1813	CAGCAAU U GGGGCCA
1388	GGGGAAU C AGUGACT	1825	CCAUGGU A CCUGCAC
1398	UGACUGU C ACUCGAG	1837	CACACCU A AAACACU
1402	UGUCACU C GAGAUCU	1845	AAACACU A GGCCACG

1856	CAAGCAU C UGAUCUG	2189	UAUUUAU U GAGUGUC
1861	AUCUGAU C UGUAGUC	2196	UGAGUGU C UUUUAUG
1865	GAUCUGU A GUCAU	2198	AGUGUCU U UUAUGUA
1868	CUGUAGU C ACAUGAC	2199	GUGUCUU U UAUGUAG
1877	CAUGACU A AGCCAAG	2200	UGUCUUU U AUGUAGG
1901	CAAGACU C AACACAU	2201	GUCUUUU A CGJAGGC
1912	ACAUGAU U GAUGGAAU	2205	UUTAUGU A GGCUAAA
1922	UGGAUGU U AAAGUCU	2210	GUAGGCU A AAUGAAC
1923	GGAUGUU A AAGUCUA	2220	UGAACAU A GGUCUCU
1928	UAAAAGU C UAGCCUG	2224	CAUAGGU C UCUGGCC
1930	AAAGUCU A GCCTGAAU	2226	UAGGUCU C UGGCCUC
1964	GAGACAU A GCCCCAC	2233	CGGGOCU C ACGGAGC
1983	AGGACAU A CAACUGG	2242	CGGAGCU C CCAGUCC
1996	GGGAAAU A CUGAAAC	2248	UCCCAGU C CAUGUCA
2005	UGAAACU U GCUGCCU	2254	UCCAUGU C ACAUCA
2013	GCUGCCU A UGGGUA	2259	GUCACAU U CRAGGUC
2015	UGCCUAU U GGGUAUG	2260	UCACAUU C AAGGUCA
2020	AUUGGGU A UGCUGAG	2266	UCAAGGU C ACCAGGU
2039	ACAGACU U ACAGAAG	2274	ACCAGGU A CAGUUGU
2040	CAGACUU A CAGAAGA	2279	GUACAGU U GUACAGG
2057	UGGCCCU C CAUAGAC	2282	CAGUUGU A CAGGUUG
2061	CCUCCAU A GACAUGU	2288	UACAGGU U GUACACU
2071	CAUGUGU A GCAUCAA	2291	AGGUGU A CRUGCA
2076	GUAGCAU C AAAACAC	2321	AAAAGAU C AAAUGGG
2097	CCACACU U CCUGACG	2338	UGGGACU U CUCAUUG
2098	CACACUU C CUCA CGG	2339	GGGACUU C UCAUUGG
2115	GCCAGCU U GGGCACU	2341	GACUCU C ADUGGCC
2128	CUGCUGU C UACUGAC	2344	UUCUCAU U GGCCAAAC
2130	GCUGUCU A CUGACCC	2358	CCUGCCU U UCCCCAG
2145	CAACCCU U GAUGAU	2359	CUGCCUU U CCCCAGA
2152	UGAUGAU A UGUAUUU	2360	UGCCUUU C CCCAGAA
2156	GAUAUGU A UUUAUUC	2376	GAGUGAU U UUUCUAU
2158	UAUGUAU U UAUUCAU	2377	AGUGAUU U UUCUAC
2159	AUGUAUU U AUUCAUU	2378	GUGAUUU U UCUAUUC
2160	UGUAUUU A UUCAUU	2379	UGAUUUU U COAU CGG
2162	UAUUUAU U CAUUGU	2380	GAUUUUU C UAU CGGC
2163	AUUUAU C AUUUGUU	2382	UUUUUCU A UCGGCAC
2166	UAUUCAU U UGUUAUU	2384	UUUCUAU C GGCACAA
2167	AUUCAUU U GUUAUU	2399	AAGCACU A UAUGGAC
2170	CAUJUGU U AUUUAAC	2401	GCACUAU A UGGACUG
2171	AUJUGUU A UUUUACC	2411	GACUGGU A AUGGUUC
2173	UUGUUAU U UUACCA	2417	UAAUGGU U CACAGGU
2174	UGUUAUU U UACCAGC	2418	AAUGGUU C ACAGGUU
2175	GUUUAUU U ACCAGCU	2425	CACAGGU U CAGAGAU
2176	UUAAJUUU A CCAGCUA	2426	ACAGGUU C AGAGAUU
2183	ACCAGCU A UUUAUUG	2433	CAGAGAU U ACCCAGU
2185	CAGCUAU U UAUUGAG	2434	AGAGAUU A CCCAGUG
2186	AGCUAUU U AUUGAGU	2448	GAGGCCU U AUUCCUC
2187	GCUAAUU A UUGAGUG	2449	AGGCCUU A UUCCUCC

2451	GCCUUAU U CCUCUCCU	2750	UAUGUGU A GACAAGC
2452	CCUUUAU C CUCCCCU	2759	ACAAGCU C UCGCUCU
2455	UAUUCU C CCUUUCOC	2761	AAGCUCU C GCUCUGU
2459	CCUCCCU U CCCCCCA	2765	UCUCGCU C UGUCACC
2460	CUCCCUU C CCCCCAA	2769	GCUCUGU C ACCCAGG
2479	GACACCU U UGUUAGC	2797	GUGCAAU C AUGGUUC
2480	ACACCUU U GUUAGCC	2803	UCAUGGU U CACUGCA
2483	CCUUUGU U AGCCACC	2804	CAUGGUU C ACUGCAG
2484	CUUJGUU A GCCACCU	2813	CUJGAGU C UUGACCU
2492	GCCACCU C CCCACCC	2815	GCAGUCU U GACCUUU
2504	CCCACAU A CAUUCU	2821	UUGACCU U UGGGGCU
2508	CAUACAU U UCUGCCA	2822	UGACCUU U UGGGCUC
2509	ATACAUU U CUGCCAG	2823	GACCUUU U GGGCUCA
2510	UACAUUU C UGCCAGU	2829	UUGGGCU C AAGUGAU
2520	CCAGUGU U CACAUG	2837	AAGUGAU C CUCCCAC
2521	CAGUGUU C ACAAUGA	2840	UGAUCCU C CCACCUC
2533	UGACACU C AGOGGUC	2847	CCCACCU C AGCCUCC
2540	CAGCGGU C AUGUCUG	2853	UCAGCCU C CUGAGUA
2545	GUCAUGU C UGGACAU	2860	CCUGAGU A GCUGGGA
2568	AGGGAAU A UGCCCAA	2872	GGACCAU A GGCUCAC
2579	CCAAGCU A UGCCUUG	2877	AUAGGCU C ACAACAC
2585	UAUGGCCU U GUCCUCU	2899	GGCAAAU U UGAJUUU
2588	GCCUUGU C CUCUUGU	2900	GCAAAUU U GAJUUUU
2591	UUGGCCU C UUGGUCC	2904	AUUGGAU U UUUUUUU
2593	GUCCUCU U GUCCUGU	2905	UUTGAUU U UUUUUUU
2596	CUCUUGU C CUGJUUG	2906	UUGAUUU U UUUUUUU
2601	GUCCUGU U UGCAUUU	2907	UGAUUUU U UUUUUUU
2602	UCCUGUU U GCAUUUC	2908	GAUUUUU U UUUUUUU
2607	UUUGCAU U UCACUGG	2909	AUUUUUU U UUUUUUU
2608	UUGCAUU U CACUGGG	2910	UUUUUUU U UUUUUUU
2609	UGCAUUU C ACUGGGA	2911	UUUUUUU U UUUUUUU
2620	GGGAGCU U GCACUAU	2912	UUUUUUU U UUUUUUUC
2626	UUGCACU A UUGCAGC	2913	UUUUUUU U UUUUUCA
2628	GCACUAU U GCAGCUC	2914	UUUUUUU U UUUUCAG
2635	UGCAGCU C CAGUUUC	2915	UUUUUUU U UUUCAGA
2640	CUCCAGU U UCCUGCA	2916	UUUUUUU U UUCAGAG
2641	UCCAGUU U CCUGCAG	2917	UUUUUUU U UCAGAGA
2642	CCAGUUU C CUGCAGU	2918	UUUUUUU U CAGAGAC
2653	CAGUGAU C AGGGUCC	2919	UUUUUUU C AGAGACG
2659	UCAGGGU C CUGCAAG	2931	ACGGGGU C UCGAAC
2689	CCAAGGU A UUGGAGG	2933	GGGGUCU C GCAACAU
2691	AAGGUAU U GGAGGAC	2941	GCAACAU U GCCCAGA
2700	GAGGACU C CCUCCCA	2951	CCAGACU U CCUUUGU
2704	ACUCCCCU C CCAGCUU	2952	CAGACUU C CTTUGUG
2711	CCCAGCU U UGGAAGG	2955	ACUUCCU U UGUGUUA
2712	CCAGCUU U GGAAGGG	2956	CUUCCUU U GUGUJAG
2721	GAAGGGU C AUCCGCG	2961	UUUGUGU U AGUAAAU
2724	GGGUCAU C CGCGUGU	2962	UUGUGUU A GUUAAUA
2744	UGUGUGU A UGUGUAG	2965	UGUUAGU U AAUAAAG

2966	GUUAGUU A AUAAAGC
2969	AGUUUAU A AAGCUUU
2975	UAAAAGCU U UCUCAAC
2976	AAGCUU U CUCAACU
2977	AAGCUUU C UCAACUG
2979	GCUUUCU C AACUGCC

Table 3

Mouse ICAM HH Target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
--------------	-----------------	--------------	-----------------

11	CCCugGU C aCCGuUG	367	AAugGCU u cAACCCcg
23	CaGuGgU u CUCUGCU	374	gAAgCCU U CCUGccC
26	uGgUuCU C UGCUCcU	375	AAgCCUU C CUgCcCCc
31	CUCUGCU c CUCCaca	378	CuacCaU C ACCGUGU
34	UuCUCaU a AGgGUcG	386	ACCGUGU A uUcGuuU
40	gCACAcU U GuAgCCU	394	CcGGACU u ucGAuCu
48	aggACCU C AGCCUGG	420	CAcCaUu C CCCcCcgg
54	UggGCCU C GugAUGG	425	CaCCCCU C ccaGCAg
58	CaUgcCU u UaGCUCC	427	CagCUCU c aGCAGug
64	cAcccCU C CCAGCAG	450	AGgACCU c ACCCUGc
96	CucugCU C CUGGccc	451	GAAaCcU u uCCJuuG
102	UgCcaGU a CUGGUgG	456	UUACCCU c aGCcaCu
108	cuCUGCU C cuGGCcC	495	CuAcCaU C ACCGUGU
115	uGGGuuCU C UGcUCCu	510	UGCUGCU C CGUGGGG
119	GgaaUGU c aCCAGGA	564	CUCAGGU a uCcAUcC
120	CUCUGcU C UggGccC	592	GAaAGAU C ACaugGG
146	CAGuCgU C cGcuUCC	607	AGCCAAU U UCUCaUG
152	UCUGUGU C agCCaCu	608	GCCAAUU U CUCaUGC
158	UCCUguU u AAAAacc	609	CCAAUUU C UCauGCC
165	CAgAAGU u gUuuUGC	611	AAUUUCU C aUGGCC
168	AAGCcUu C CUGCCCC	656	aAGCUGU U UGAGcug
185	GGuGGgU C CGUGCAG	657	AGCUGUU U GAGcuga
209	gcCACUu C CUCUGgc	668	cgagCCU a GGCCaCC
227	C2gAAGU U GUUuuUGC	677	GaCCuCU A CCAGCcU
230	AAGUUGU U uuGCuCC	684	uuCAGCU C CgGuCCU
237	UGuGCuU u GAGAaCu	692	CgGACUu U cGauCUu
248	AaCCCaU c uCCUAAA	693	AGgaCcU c acCCUGC
253	ccUGCCU A AggAaGA	696	CCUgUuU C CUGCCuc
263	AgGGuuU c uCUaCUG	709	gGCCgCU C CaCCuCA
267	AGggGCCU C CUGCCua	720	uACAACU U uUCAGCu
293	AAGcUGU u UGAgCUG	723	AAUuUuU C AGCuCCg
319	AGgAGAU A cugAgCC	735	aCCaGaU C CUGGAGa
335	cUGUGCU u UgagAAC	738	uGGgCCU c GuGaUGG
337	GUcCaAU U CACACUG	765	CaGUcGU C cGcUuCC
338	aGCUgUU u gAgCUGa	769	GGcCUGU U uCCUGcc
359	GuGCAGU C guCcGCC	770	uUuUGcU C CCUGGAa
785	GGcCUGU U uCCUgCc	1353	AGUGGgU c gAAgGgUG
786	GcCUGUU u CCuGcCU	1366	UaaCAgU c UaCaACU
792	UggagGU C UCGGAaG	1367	aGCACcU c CCCACCU
794	CugGgCU u GGAGaCu	1368	GuACUgU a CCACUcu
807	CuCgGaU a uACCUGG	1380	UGCCCAU C GGGGugg
833	CAaAGcU c GAcaCCC	1388	GGaGAcU C AGUGgCU
846	CCcugGU C ACCguUG	1398	UGgCUGU C ACagaAc
851	GagACCU c UacCAgC	1402	UGUgCUU u GAGAaCU

863	AgCcACU u CcUCUgG	1408	gCGAGAU C ggGgaGG
866	GAagCCU U CcuGcCC	1410	GAGgUCU c GgaaGgg
867	AuUCgUU u cCGGagA	1421	cccACCU A CuUuUGU
869	UCuUcCU C augCAAG	1425	aCUGCCU u gGUaGaG
881	AuGGCuU C AacCcGU	1429	uCUCUaU u GccCCuG
885	CCUugGU a gagGUGA	1444	GAaggCU C AgGaGGA
933	cUauAaU c AUuCTGG	1455	GGaAuGU C ACCaGga
936	uAaUcAU u CUGGUgC	1482	AguUGuU u UgCuCCC
978	UaACagU C UACAAzCU	1484	cUGuUCU u CCuCauG
980	ACagUCU A CAaCUUU	1493	CuguGcU u UGAGAac
986	UACAAaCU U UuCaGCU	1500	AUGAaAU c aUggUCc
987	ACAaCUU U uCaGCUc	1503	gGAcUaU a AUCAUuc
988	CAaCUUU u CaGCuCC	1506	UuaUguU u AUaACcG
1005	ACcaGAU c CUGgagaA	1509	cuAcCAU C ACCGUGu
1006	uGaGAgU C UGggGAA	1518	ucaUGGU c cCAGgCG
1023	ugGAGGU C UCgGAAG	1530	CuaaAaU C AUucUGG
1025	GAGGUcU C gGAAGGG	1533	ugGUCAU u gUGGGCC
1066	CCACUcU c aAaaauAA	1551	CAuGCCU u AGCAgcu
1092	AcuGGaU c uCAGgCC	1559	AGCACCU c CCCaccU
1093	UGGaccU u CAGGCaA	1563	CuUAugU u UAUAACC
1125	CCCAsCU C uUcuUCA	1565	UAugUuU A UAACCGC
1163	CGaAGCU .U CUuUUGC	1567	ugUuUAU A ACCGCCA
1164	GaAGCUU C UuuUGCU	1584	GaAAAGAU C AgGAuAU
1166	AGCUUCU u uUGCUCU	1592	AgGAuAU A CAaguUA
1172	UCCUGuU u aaaAACC	1599	ACAAguU A CAgAAgg
1200	cuCuGCU c cUcCACA	1651	CcCaCCU C CCUGAgC
1201	gCuGCUU u UgaACAG	1661	gaAACCU u UCCuuuG
1203	AcuUUuU u CACcAGu	1663	AAACCUuU C CuuuGAa
1227	GGuAcaU a CGUGUgC	1678	AGGaCCU C agCCUgG
1228	GaAGCUU C uUuUgCU	1680	aGCCaCU U CCUCuGg
1233	UUCGUuU C CgGagaG	1681	GCCaCUU C CUCuGgC
1238	GUGcUGU A UGGuCCU	1684	acUJUCCU C uGgCUgu
1264	GAaGGgU c GUGCaAG	1690	cCGGaCU U uCgAUcU
1267	uGAgA GU C uGGGgAA	1691	CGGacUU u CgAUcUU
1294	AGgAgAU a CugAGCc	1696	UgCCCCAU c ggGGUGG
1295	GAggggU C uCAGCAG	1698	CggAUAU a ccUggag
1306	GCAGACU C ugAaaUG	1737	gAGACCU c UaCCAgc
1321	gaAGGCU c aGGaGgA	1750	gGCgGCU c CACCUca
1334	AACCCAU c uCCuaAA	1756	gAagCCU u CCuGCC
1344	auGAGCU C gAGaGUg	1787	gaGaCAU U GUCCcCA
1351	ugAaUGU a UAAguua	1790	GCAUUGU u CUCuaau
1793	UgGUCCU C gGcugGA	2173	UUagagU U UUACCAG
1797	CacCAGU C AcAUAAaA	2174	UagagUU U UACCAgC
1802	acCAGAU c CuggAGa	2175	agagUUU U ACCAGCU
1812	ACuGgAU c UcaGGCC	2176	gagUUUU A CCAGCUA
1813	CAGCAUU U acccuCA	2183	ACCAGCU A UUUUuUG
1825	CCAcGcU A CCUcugC	2185	CAGCUAU U UAUUGAG
1837	CAugCCU u uAgCuCc	2186	AGCUAUU U AUUGAGU
1845	cgAgcCU A GGCCACc	2187	GUUAUUU A UUGAGUa

1856	Cgg <u>a</u> CuU u cGAUCUu	2189	UAUUUAU U GAGUacC
1861	A <u>c</u> UGAU a UccAGU <u>a</u>	2196	caAcUcU u cUUgAUG
1865	c <u>Ac</u> uUGU A G <u>c</u> U <u>c</u> Ag	2198	gcaGcCU c UUAUGUu
1868	CaccAGU C ACAU <u>a</u> Aa	2199	GccUCUU a UgUuUAu
1877	CAUGcCU u AGCagcu	2200	UcUuccU c AUGcAaG
1901	uAA <u>a</u> ACU C AAGggAc	2201	aagUUUU A UGU <u>c</u> GGC
1912	AuAU <u>ag</u> U a GAU <u>c</u> agU	2205	UUUAUGU c GGC <u>c</u> ugA
1922	UGaAUGU a uAAGGU <u>a</u>	2210	GgAGaCU c AgUGgcu
1923	uGAUGcU c AgGU <u>a</u> Uc	2220	cuggCAU u GuUCUCU
1928	UU <u>Ag</u> AGU u UuaCCaG	2224	CucAGGU a UC <u>cau</u> CC
1930	A <u>g</u> AGUuU u aCCaGcU	2226	UgGaUCU C aGGCCgC
1964	GAGAC <u>a</u> U u GuCCC <u>a</u>	2233	CJG <u>a</u> CCJ C cuGGAGg
1983	AGGA <u>uu</u> AU A CA <u>Ag</u> U <u>ua</u>	2242	uGGAGCU a gCgGaCC
1996	aGG <u>Ag</u> AU A CUG <u>Ag</u> CC	2248	UauCc <u>a</u> U C CAU <u>cc</u> CA
2005	UGgAgCU a GC <u>g</u> GaCc	2254	UCC <u>a</u> auU C AC <u>Ac</u> UgA
2013	GC <u>U</u> auuuU A UUG <u>a</u> GU <u>a</u>	2259	aUCACAU U C <u>Ac</u> GGU <u>g</u>
2015	UGGCC <u>a</u> U c GGG <u>g</u> ugG	2260	UCACAUU C AcGGU <u>g</u> c
2020	ggUGGU <u>a</u> U u <u>u</u> CUGAG	2266	ggAA <u>u</u> GU C ACCAGGA
2039	gCuGgCU a gCAG <u>Ag</u> G	2274	ACCAG <u>a</u> U c CuG <u>g</u> GA <u>g</u>
2040	CuGAC <u>c</u> U c CuGg <u>Ag</u> g	2279	GaAg <u>g</u> GU c GU <u>g</u> CA <u>a</u> G
2057	UG <u>cu</u> CCJ C C <u>Ac</u> A <u>u</u> C	2282	aAG <u>c</u> UGU u ugaG <u>c</u> UG
2061	C <u>u</u> uCCAU c ac <u>Cg</u> UGU	2288	UAu <u>a</u> GU U aUgg <u>c</u> CU
2071	CA <u>c</u> uUGU A GC <u>c</u> U <u>c</u> Ag	2291	caGU <u>g</u> GU u CuCUG <u>U</u>
2076	GUAGC <u>c</u> U C Ag <u>Ag</u> C <u>u</u> a	2321	gAA <u>AG</u> AU C AcAU <u>GG</u> G
2097	CaAC <u>u</u> CU U CuUG <u>Gu</u> uG	2338	UG <u>Ag</u> ACU c CU <u>ge</u> cUG
2098	CACACUU C C <u>ccc</u> C <u>cg</u>	2339	GaaAC <u>c</u> U u UC <u>c</u> UU <u>u</u> G
2115	GCCAGCU c GGagg <u>g</u> au	2341	GAC <u>c</u> UCU a c <u>ca</u> G <u>c</u> U
2128	CaG <u>CU</u> uU u UA <u>u</u> U <u>g</u> AG	2344	UU <u>u</u> cgAU c uuCC <u>Ag</u> C
2130	c <u>CU</u> GU <u>u</u> U c CUG <u>c</u> U <u>c</u>	2358	CC <u>c</u> agCU c UC <u>ag</u> C <u>AG</u>
2145	CAA <u>CU</u> U U cuUG <u>Gu</u> uG	2359	CUG <u>Gu</u> UU U gaaCAGA
2152	Uau <u>U</u> aAU u U <u>ag</u> Ag <u>GU</u>	2360	aaCCUUU C C <u>uuu</u> GAA
2156	uugAUGU A UUU <u>uu</u> uA	2376	agGU <u>GG</u> GU U cuUCU <u>g</u> a
2158	gAUG <u>GU</u> U U UAU <u>u</u> A <u>U</u>	2377	gGU <u>GG</u> GUU c UUCU <u>g</u> ag
2159	AUG <u>GU</u> UU U AUU <u>a</u> AU <u>U</u>	2378	agG <u>g</u> UUU c UCU <u>Ac</u> uG
2160	UG <u>GU</u> UUU A UU <u>a</u> UU <u>U</u>	2379	UG <u>GU</u> UUU c ucAU <u>aa</u> G
2162	U <u>AU</u> UUU U aAUU <u>U</u> u <u>g</u>	2380	aAg <u>UU</u> UU a UgUCGGC
2163	AU <u>g</u> U <u>AU</u> U u AUU <u>aa</u> U <u>U</u>	2382	aUU <u>c</u> UCU A UuG <u>c</u> CC
2166	ac <u>UU</u> CAU U cuc <u>U</u> AU <u>U</u>	2384	aU <u>c</u> C <u>ag</u> U a GaCACAA
2167	AU <u>gu</u> U <u>U</u> U u aUU <u>U</u> u <u>U</u>	2399	A <u>AA</u> AC <u>U</u> A UgUGGAC
2170	U <u>AU</u> UU <u>U</u> U u A <u>uu</u> U <u>U</u> u <u>g</u>	2401	aag <u>C</u> U <u>g</u> U u DG <u>ag</u> CUG
2171	A <u>g</u> U <u>U</u> GU <u>U</u> u U <u>g</u> c <u>U</u> c <u>CC</u>	2411	uACUGGU c Ag <u>g</u> aU <u>g</u> C
2417	gAA <u>U</u> GGGU a CA <u>u</u> Ac <u>GU</u>	2691	AA <u>u</u> GU <u>c</u> U c CGAGG <u>g</u> C
2418	Ac <u>U</u> GG <u>g</u> U C u <u>C</u> AGG <u>g</u> cc	2700	GA <u>a</u> G <u>c</u> U u CC <u>U</u> g <u>CC</u> C
2425	CA <u>u</u> ggGU c g <u>Ag</u> g <u>Gu</u> U	2704	g <u>ac</u> C <u>U</u> U a CCAG <u>C</u> U
2426	A <u>uu</u> aa <u>U</u> U u AG <u>Ag</u> U <u>U</u>	2711	CC <u>CA</u> GU c U <u>c</u> ag <u>ca</u> G
2433	uAG <u>AG</u> GU <u>U</u> U uaCC <u>AG</u> U <u>c</u>	2712	g <u>ag</u> GU <u>c</u> U c G <u>GA</u> AGGG
2434	AG <u>AG</u> GU <u>U</u> U aCC <u>AG</u> U <u>c</u>	2721	GA <u>AG</u> GGGU C g <u>U</u> g <u>C</u> aaG
2448	GA <u>a</u> G <u>CC</u> U U cc <u>U</u> g <u>CC</u> CC	2724	GG <u>ua</u> CAU a CG <u>u</u> GU <u>g</u> C
2449	A <u>a</u> G <u>CC</u> UU c c <u>U</u> g <u>CC</u> CC	2744	gGU <u>GG</u> GU c c <u>GU</u> g <u>C</u> AG

2451	GCCUguU	U	CCUgCCC	2750	UAUuUaU	u	GAguaCc
2452	CCUguUU	C	CUgCCUC	2759	cCggacU	u	UCGaUCU
2455	gAagCCU	u	CCUgCCC	2761	AgGaccU	C	aCcCUGc
2459	CCaCaCU	U	CCCCCCCC	2765	UuUugCU	C	UGcCgCu
2460	CaCaCUU	C	CCCCCCCg	2769	agUCUGU	C	AaaCAGG
2479	GAgACCU	c	UaccAGC	2797	aUG2AAU	C	AUGGUccC
2480	uCACCgU	U	GUgAuCC	2803	UCAUGGU	c	CcagGCg
2483	CCaaUGU	c	AGCCACC	2804	ggUGGGgU	C	cgUGCAG
2484	CUUUUUU	c	aCCAguc	2813	CUccGGU	C	cUGACCC
2492	agCACCU	C	CCCACCU	2815	aCAGUCU	a	caaCUUU
2504	CCCACcU	A	CUUUUGU	2821	cUGACCU	c	cUGGagg
2508	uAUcCAU	c	caUcCCA	2822	gGAgCcU	c	cGGaCUu
2509	uUAgAgU	U	uUaCCAG	2823	ugCCUUU	a	GcuCcCA
2510	UAgAgUU	u	UaCCAGC	2829	cUGG2CU	a	uA2UcAU
2520	CuuuUGU	U	CcCAAUG	2837	AgGUGgU	u	CUuCuGA
2521	CAGcaUU	u	ACccUCA	2840	UGAgacU	C	CugCCUg
2533	UGAugCU	C	AGguaUC	2847	CCaAugU	C	AGCCaCC
2540	CAGCaGU	C	cgcUgUG	2853	gCAGCCU	C	uUauGUu
2545	GUgcUGU	a	UGGuCcU	2860	gCcaAGU	A	acUGuGA
2568	guGaAgU	c	UGuCaAA	2872	GGACCUU	c	aGCcaAg
2579	auAAGUu	A	UGgCcUG	2877	uUccGCU	a	cCAuC2C
2585	cugGCaU	U	GUuCUCU	2899	cGgAcuU	U	cGAUcUU
2588	GCaUUGU	u	CUCUaaU	2900	uuAAuUU	a	GAgUUUU
2591	UgGUuCU	C	VgcUCCU	2904	AcUTcAU	U	cUcUaUU
2593	cUuCUuU	U	GcuCUGC	2905	cUUcAUU	c	UcUaUUG
2596	CUuUUGU	u	CccaaUG	2906	UUGAUgU	a	UUUaUUa
2601	acCgUGU	a	UuCgUUU	2907	UGuaUUU	a	UUaaUUU
2602	UCCaGcU	a	cCAUccc	2908	GAagcUU	c	UUUUGcU
2607	cUcGgAU	a	UacCUGG	2909	AgcUUeU	U	UUgcUcU
2608	caGCAgU	c	CgCUGuG	2910	UgUaUUU	a	UUaaUUU
2609	gGaAUgU	C	ACcaGGA	2911	UgUaUUU	a	UUaaUUU
2620	AGGAcCU	c	aCcCUGc	2912	UUgUUcU	c	UaaUgUC
2626	UJUuCgaU	c	UJccCAGC	2913	UUUcUcU	a	cUggUCA
2628	GCACacU	U	GuAGCcU	2914	UgcUUUU	c	UcaUaAG
2635	UuCAGCU	C	CgGGUccU	2915	aUUUJaUU	a	aUUuAGA
2640	ggCCuGU	U	UCCUUGCc	2916	UaUUcgU	U	UcCgGAG
2641	CCCAGcU	c	uCaGCAG	2917	aUUUcgUU	U	cCgGAGA
2642	CCuGUUU	C	CUGCcuc	2918	UUcgUUU	c	CgGAGAg
2653	uAcUGgU	C	AGGaUgC	2919	UUCUcaU	a	AGgGuCG
2659	gaAGGGU	C	gUGCAAG	2931	ugGAGGU	C	UCGgAAg
2689	CuAAuGU	c	UccGAGG	2933	GaGGUCU	C	GgAAggg
2941	GagACAU	U	GuCCccA				
2951	CCAcgCU	a	CCUcUGC				
2952	CAGcagU	C	CgcUGUG				
2955	AgUgaCU	c	UGUGUcA				
2956	uUUCCUU	U	GaaUcAa				
2961	UcUGUGU	c	AGccAcU				
2962	aUGUaUU	u	aUUAAUu				
2965	UuUgAaU	c	AAUAAAG				

2966	GcUgGcU A gcAgAGg
2969	AaUcAAU A AAQuUU
2975	UAgAGuU U UacCAGC
2976	gAgGgUU U CUQuACU
2977	AAGCUgU u UgAgCUG
2979	uCaUUUCU C uAuUGCC

Table 4
Human ICAM HH Ribozyme Sequences

nt. Position	Ribozyme Sequence
11	CAGCGUC CUGAUGAGGCCGAAAGGCCGAA ACUGGGG
23	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCUCAG
26	A GUAGCA CUGAUGAGGCCGAAAGGCCGAA AGGACCU
31	CUCUGAG CUGAUGAGGCCGAAAGGCCGAA ACCAGAG
34	CAACUCU CUGAUGAGGCCGAAAGGCCGAA AGTACCA
40	AGGUUCG CUGAUGAGGCCGAAAGGCCGAA ACUCUGA
48	CGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUUGC
54	CCAUAGC CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
58	GGAGCCA CUGAUGAGGCCGAAAGGCCGAA AGCGAGG
64	CUGCCUG CUGAUGAGGCCGAAAGGCCGAA AGCCAU
96	GGACCCAG CUGAUGAGGCCGAAAGGCCGAA AGUGCGG
102	CGAGCCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGA
108	GAGCCCC CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
115	GGGAACA CUGAUGAGGCCGAAAGGCCGAA AGCCCCG
119	UCCUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGAGC
120	GUCCUGG CUGAUGAGGCCGAAAGGCCGAA AACAGAG
146	GGACACA CUGAUGAGGCCGAAAGGCCGAA AUGUCUG
152	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA ACACAGA
158	GACUUUU CUGAUGAGGCCGAAAGGCCGAA AGGGGGA
165	GCAGGAAU CUGAUGAGGCCGAAAGGCCGAA ACUUUUG
168	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AUGACUU
185	CAGCACG CUGAUGAGGCCGAAAGGCCGAA AGCCUCC
209	GUCACAG CUGAUGAGGCCGAAAGGCCGAA AGGUGCU
227	GCCCACAC CUGAUGAGGCCGAAAGGCCGAA ACUUGGG
230	UAUUCCC CUGAUGAGGCCGAAAGGCCGAA ACAACTUU
237	GGGUCUC CUGAUGAGGCCGAAAGGCCGAA AUGCCCA
248	UUUAGGC CUGAUGAGGCCGAAAGGCCGAA ACGGGGU
253	UCCUUUU CUGAUGAGGCCGAAAGGCCGAA AGGCAAC
263	CAGGAGC CUGAUGAGGCCGAAAGGCCGAA ACUCCUU
267	CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAACU
293	CAGUUCA CUGAUGAGGCCGAAAGGCCGAA ACACCUU
319	GGUUGGC CUGAUGAGGCCGAAAGGCCGAA AUUUUCU
335	GUUUGAA CUGAUGAGGCCGAAAGGCCGAA AGCACAU
337	CAGUUUG CUGAUGAGGCCGAAAGGCCGAA AUAGCAC
338	GCAGUUU CUGAUGAGGCCGAAAGGCCGAA AAUAGCA
359	AGCUGUU CUGAUGAGGCCGAAAGGCCGAA ACUGCCC
367	AAGGUUU CUGAUGAGGCCGAAAGGCCGAA AGCUGUU
374	GGUGAGG CUGAUGAGGCCGAAAGGCCGAA AGGUUUU
375	CGGUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGUUU
378	ACACGGU CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
386	AGUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACGGU
394	CGUUCUG CUGAUGAGGCCGAAAGGCCGAA AGUCCAG
420	AAGAGGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG
425	CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AGGGGAG

427 GGCUGCC CUGAUGAGGCCGAAAGGCCGAA AGAGGGG
 450 GUAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUUCU
 451 CGUAGGG CUGAUGAGGCCGAAAGGCCGAA AAGGUUC
 456 GGCAGCG CUGAUGAGGCCGAAAGGCCGAA AGGGUAA
 495 CCACGGU CUGAUGAGGCCGAAAGGCCGAA AGGUUGG
 510 CCCCACG CUGAUGAGGCCGAAAGGCCGAA AGCAGCA
 564 UGGUCGU CUGAUGAGGCCGAAAGGCCGAA ACCUCAG
 592 CCAUGGU CUGAUGAGGCCGAAAGGCCGAA AUCUCUC
 607 CACGAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGCU
 608 GCACGAG CUGAUGAGGCCGAAAGGCCGAA AAUUGGC
 609 GGCACGA CUGAUGAGGCCGAAAGGCCGAA AAAUUGG
 611 GGGCAC CUGAUGAGGCCGAAAGGCCGAA AGAAAUU
 636 GUUCUCA CUGAUGAGGCCGAAAGGCCGAA ACAGCUC
 657 UGUUCUC CUGAUGAGGCCGAAAGGCCGAA AACAGCU
 668 GGGGCC CUGAUGAGGCCGAAAGGCCGAA AGGUGUU
 677 GAGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGGC
 684 AGGUCUG CUGAUGAGGCCGAAAGGCCGAA AGCUGGU
 692 CAGGACA CUGAUGAGGCCGAAAGGCCGAA AGGUUCG
 693 GCAGGAC CUGAUGAGGCCGAAAGGCCGAA AAGGUCU
 696 CUGGCAG CUGAUGAGGCCGAAAGGCCGAA ACAAAAGG
 709 UGUGGGG CUGAUGAGGCCGAAAGGCCGAA AGUCGCU
 720 GGCUGAC CUGAUGAGGCCGAAAGGCCGAA AGUUGUG
 723 GGGGGCU CUGAUGAGGCCGAAAGGCCGAA ACAAGUU
 735 CCUCUAG CUGAUGAGGCCGAAAGGCCGAA ACCCGGG
 738 CCACCUU CUGAUGAGGCCGAAAGGCCGAA AGGACCC
 765 GGGAAACA CUGAUGAGGCCGAAAGGCCGAA ACCACGG
 769 UCCAGGG CUGAUGAGGCCGAAAGGCCGAA ACAGACC
 770 GUCCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGAC
 785 GACUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGCCC
 786 AGACUGG CUGAUGAGGCCGAAAGGCCGAA AACAGCC
 792 CCUCCGA CUGAUGAGGCCGAAAGGCCGAA ACUGGGA
 794 GGCCUCC CUGAUGAGGCCGAAAGGCCGAA AGACUGG
 807 CCAGGUG CUGAUGAGGCCGAAAGGCCGAA ACCUGGG
 833 GGGGUUC CUGAUGAGGCCGAAAGGCCGAA ACCUCUG
 846 CAUAGGU CUGAUGAGGCCGAAAGGCCGAA ACUGUGG
 851 GUUGCCA CUGAUGAGGCCGAAAGGCCGAA AGGUGAC
 863 CGAGAAC CUGAUGAGGCCGAAAGGCCGAA AGUCGUU
 866 GGGCGAG CUGAUGAGGCCGAAAGGCCGAA AGGAGUC
 867 UGGCCGA CUGAUGAGGCCGAAAGGCCGAA AAGGAGU
 869 CUUGGCC CUGAUGAGGCCGAAAGGCCGAA AGAAGGA
 881 ACUGACU CUGAUGAGGCCGAAAGGCCGAA AGGCCUU
 885 UCACACU CUGAUGAGGCCGAAAGGCCGAA ACUGAGG
 933 CCAGUAU CUGAUGAGGCCGAAAGGCCGAA ACUGCAC
 936 UCCCCAG CUGAUGAGGCCGAAAGGCCGAA AUUACUG
 978 AGCUGUA CUGAUGAGGCCGAAAGGCCGAA AUGGUCA
 980 AAAGCUG CUGAUGAGGCCGAAAGGCCGAA AGAUGGU
 986 CGCCCGA CUGAUGAGGCCGAAAGGCCGAA AGCUGUA
 987 GCGCCGG CUGAUGAGGCCGAAAGGCCGAA AAGCUGU
 988 GCGCCCG CUGAUGAGGCCGAAAGGCCGAA AAAGCUG

1005 UCGUCAG CUGADGAGGCCGAAAGGCCGAA AUCACGU
 1006 UUCGUCA CUGAUGAGGCCGAAAGGCCGAA AAUCACG
 1023 CUUCUGA CUGADGAGGCCGAAAGGCCGAA ACCUCUG
 1025 CCCUUCU CUGAUGAGGCCGAAAGGCCGAA AGACCUUC
 1066 UGGGCUC CUGAUGAGGCCGAAAGGCCGAA AGGGUGG
 1092 GGGCUGG CUGADGAGGCCGAAAGGCCGAA ACCCCAU
 1093 UGGGCUG CUGAUGAGGCCGAAAGGCCGAA AACCCCA
 1125 UCAGCAG CUGAUGAGGCCGAAAGGCCGAA ACCUGGG
 1163 GCAGGGAG CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
 1164 AGCAGGA CUGAUGAGGCCGAAAGGCCGAA AAGCUGC
 1166 AGAGCAC CUGAUGAGGCCGAAAGGCCGAA AGAACGU
 1172 GGUUGCA CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
 1200 UGUGUAU CUGAUGAGGCCGAAAGGCCGAA AGCUGGC
 1201 UUGUGUA CUGAUGAGGCCGAAAGGCCGAA AAGCUGG
 1203 UCUUGUG CUGAUGAGGCCGAAAGGCCGAA AUAAAGCU
 1227 GGACACG CUGAUGAGGCCGAAAGGCCGAA AGCUCCC
 1228 AGGACAC CUGAUGAGGCCGAAAGGCCGAA AAGCUCC
 1233 CAUACAG CUGAUGAGGCCGAAAGGCCGAA ACACGAA
 1238 GGGGCCA CUGAUGAGGCCGAAAGGCCGAA ACAGGAC
 1264 CCCGGAC CUGAUGAGGCCGAAAGGCCGAA AUCCCCUC
 1267 UUUCCCC CUGAUGAGGCCGAAAGGCCGAA ACAAUCC
 1294 UGGUGGG CUGAUGAGGCCGAAAGGCCGAA AUUUUUC
 1295 CUGUGGG CUGAUGAGGCCGAAAGGCCGAA AUUUUUC
 1306 CACAUUG CUGAUGAGGCCGAAAGGCCGAA AGUCUGC
 1321 UUCCCCC CUGAUGAGGCCGAAAGGCCGAA AGCCUGG
 1334 CUCGGGC CUGAUGAGGCCGAAAGGCCGAA AUGGGUU
 1344 GACACUU CUGAUGAGGCCGAAAGGCCGAA AGCUCGG
 1351 UCCUUUA CUGAUGAGGCCGAAAGGCCGAA ACACUUG
 1353 CAUCCUU CUGAUGAGGCCGAAAGGCCGAA AGACACU
 1366 AGUGGGG CUGAUGAGGCCGAAAGGCCGAA AGUGCCA
 1367 CAGUGGG CUGAUGAGGCCGAAAGGCCGAA AAGUGCC
 1368 GCAGUGGG CUGAUGAGGCCGAAAGGCCGAA AAAGUGC
 1380 AUUCCCC CUGAUGAGGCCGAAAGGCCGAA AUGGGCA
 1388 AGUCACU CUGAUGAGGCCGAAAGGCCGAA AUUCCCC
 1398 CUCGAGU CUGAUGAGGCCGAAAGGCCGAA ACAGUCA
 1402 AGAUCUC CUGAUGAGGCCGAAAGGCCGAA AGUGACA
 1408 CCCUCAA CUGAUGAGGCCGAAAGGCCGAA AUCUCGA
 1410 UGCCCCUC CUGAUGAGGCCGAAAGGCCGAA AGAUCUC
 1421 ACAGAGG CUGAUGAGGCCGAAAGGCCGAA AGGUGCC
 1425 CCCGACA CUGAUGAGGCCGAAAGGCCGAA AGGUAGG
 1429 CUGGGCC CUGAUGAGGCCGAAAGGCCGAA ACAGAGG
 1444 UCCCCUU CUGAUGAGGCCGAAAGGCCGAA AGUGCUC
 1455 CGGGGGU CUGAUGAGGCCGAAAGGCCGAA ACCUCCC
 1482 GGGGGGA CUGAUGAGGCCGAAAGGCCGAA AGCACAU
 1484 CGGGGGG CUGAUGAGGCCGAAAGGCCGAA AGAGCAC
 1493 AAUCUCA CUGAUGAGGCCGAAAGGCCGAA ACCGGGG
 1500 UGAUGAC CUGAUGAGGCCGAAAGGCCGAA AUCUCAU
 1503 UGAUGAU CUGAUGAGGCCGAAAGGCCGAA ACAAUCAU
 1506 CAGUGAU CUGAUGAGGCCGAAAGGCCGAA AUGACAA

1509 CCACAGU CUGAUGGCGGAAGGCCGAA AUGAUGA
 1518 CGGCUGC CUGAUGGCGGAAGGCCGAA ACCACAG
 1530 CCAUUAU CUGAUGGCGGAAGGCCGAA ACUGCGG
 1533 UGCCCAU CUGAUGGCGGAAGGCCGAA AUGACUG
 1551 ACGUGCU CUGAUGGCGGAAGGCCGAA AGGCCUG
 1559 AUAGAGG CUGAUGGCGGAAGGCCGAA ACGUGCU
 1563 GGUUAUA CUGAUGGCGGAAGGCCGAA AGGUACG
 1565 GCGGUUA CUGAUGGCGGAAGGCCGAA AGAGGUAA
 1567 UGGCGGU CUGAUGGCGGAAGGCCGAA AUAGAGG
 1584 AUUUUUU CUGAUGGCGGAAGGCCGAA AUCUUCC
 1592 UAGUCUG CUGAUGGCGGAAGGCCGAA AUUUUUU
 1599 CCUGUUG CUGAUGGCGGAAGGCCGAA AGUCUGU
 1651 GUUCAGG CUGAUGGCGGAAGGCCGAA AGGCGUG
 1661 CCCGGGA CUGAUGGCGGAAGGCCGAA AGGUUCA
 1663 GUCCCCG CUGAUGGCGGAAGGCCGAA AUAGGUU
 1678 CGAGGAA CUGAUGGCGGAAGGCCGAA AGGCCCU
 1680 GCGGAGG CUGAUGGCGGAAGGCCGAA AGAGGCC
 1681 GGGCGAG CUGAUGGCGGAAGGCCGAA AAGAGGC
 1684 GAAGGCC CUGAUGGCGGAAGGCCGAA AGGAAGA
 1690 AUAUGGG CUGAUGGCGGAAGGCCGAA AGGCCGA
 1691 AAUAUGG CUGAUGGCGGAAGGCCGAA AAGGCCG
 1696 CCACCAA CUGAUGGCGGAAGGCCGAA AUGGGAA
 1698 UGCCACC CUGAUGGCGGAAGGCCGAA AUAUGGG
 1737 CAUGGCA CUGAUGGCGGAAGGCCGAA AUGUCUU
 1750 GUAGGUG CUGAUGGCGGAAGGCCGAA AGCUGCA
 1756 GGGCGGG CUGAUGGCGGAAGGCCGAA AGGUGUA
 1787 UGAGGAC CUGAUGGCGGAAGGCCGAA AUGCCCU
 1790 GACUGAG CUGAUGGCGGAAGGCCGAA ACAAUGC
 1793 UCUGACU CUGAUGGCGGAAGGCCGAA AGGACAA
 1797 UGUAAUCU CUGAUGGCGGAAGGCCGAA ACUGAGG
 1802 GCUGUUG CUGAUGGCGGAAGGCCGAA AUCUGAC
 1812 GGGCCCC CUGAUGGCGGAAGGCCGAA AUGCUGU
 1813 UGGCCCC CUGAUGGCGGAAGGCCGAA AAUGCTUG
 1825 GUCCAGG CUGAUGGCGGAAGGCCGAA ACCADGG
 1837 AGUGUUU CUGAUGGCGGAAGGCCGAA AGGUGUG
 1845 CGUGGCC CUGAUGGCGGAAGGCCGAA AGUGUUU
 1856 CAGAUCA CUGAUGGCGGAAGGCCGAA AUGCGUG
 1861 GACUACA CUGAUGGCGGAAGGCCGAA AUCAGAU
 1865 AUGUGAC CUGAUGGCGGAAGGCCGAA ACAGAUC
 1868 GUCAUGU CUGAUGGCGGAAGGCCGAA ACUACAG
 1877 CUUGGCCU CUGAUGGCGGAAGGCCGAA ACUACAG
 1901 AUGUCUU CUGAUGGCGGAAGGCCGAA AGUCAUG
 1912 AUCCAUC CUGAUGGCGGAAGGCCGAA AUCAUGU
 1922 AGACUUU CUGAUGGCGGAAGGCCGAA ACACUCCA
 1923 UAGACUU CUGAUGGCGGAAGGCCGAA AACAUCC
 1928 CAGGCUA CUGAUGGCGGAAGGCCGAA ACUUUAA
 1930 AUCAGGC CUGAUGGCGGAAGGCCGAA AGACUUU
 1964 GUGGGGC CUGAUGGCGGAAGGCCGAA AUGUCUC
 1983 CCAGUUG CUGAUGGCGGAAGGCCGAA AUGUCCU

1996	GUUUCAG CUGAUGAGGCCGAAAGGCCGAA AUUUCCC
2005	AGGCAGC CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
2013	UACCAA CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
2015	CAUACCC CUGAUGAGGCCGAAAGGCCGAA AUAGGCA
2020	CUCAGCA CUGAUGAGGCCGAAAGGCCGAA ACCCAAU
2039	CUUCUGU CUGAUGAGGCCGAAAGGCCGAA AGUCUG
2040	UCUUCUG CUGAUGAGGCCGAAAGGCCGAA AAGUCUG
2057	GUCUADG CUGAUGAGGCCGAAAGGCCGAA AGGGCCA
2061	ACAUGJC CUGAUGAGGCCGAAAGGCCGAA AUGGAGG
2071	UUGAUGC CUGAUGAGGCCGAAAGGCCGAA ACACAUU
2076	GUGUUUU CUGAUGAGGCCGAAAGGCCGAA AUGCUAC
2097	CGJCAGG CUGAUGAGGCCGAAAGGCCGAA AGUGUGG
2098	COGUCAG CUGAUGAGGCCGAAAGGCCGAA AAGUGUG
2115	AGUGCCC CUGAUGAGGCCGAAAGGCCGAA AGCUGGC
2128	GUCAGUA CUGAUGAGGCCGAAAGGCCGAA ACAGCAG
2130	GGGUCAG CUGAUGAGGCCGAAAGGCCGAA AGACAGC
2145	UAUCAUC CUGAUGAGGCCGAAAGGCCGAA AGGGUUG
2152	AAAUAACA CUGAUGAGGCCGAAAGGCCGAA AUCAUCA
2156	GAUAAA CUGAUGAGGCCGAAAGGCCGAA ACAUAC
2158	AUGAAUA CUGAUGAGGCCGAAAGGCCGAA AUACAUUA
2159	AAUGAAU CUGAUGAGGCCGAAAGGCCGAA AAUACAU
2160	AAAUGAA CUGAUGAGGCCGAAAGGCCGAA AAAUACA
2162	ACAAAUG CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
2163	AACAAAU CUGAUGAGGCCGAAAGGCCGAA AAUAAAUA
2166	AAUUAACA CUGAUGAGGCCGAAAGGCCGAA AUGAAUA
2167	AAAUAAC CUGAUGAGGCCGAAAGGCCGAA AAUGAAU
2170	GUAAAAAU CUGAUGAGGCCGAAAGGCCGAA ACAAAUG
2171	GGUAAAA CUGAUGAGGCCGAAAGGCCGAA AACAAAU
2173	CUGGUAA CUGAUGAGGCCGAAAGGCCGAA AUAAACA
2174	GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AAUAACA
2175	AGCUGGU CUGAUGAGGCCGAAAGGCCGAA AAAUAAC
2176	UAGCUGG CUGAUGAGGCCGAAAGGCCGAA AAAUAA
2183	CAAUAAA CUGAUGAGGCCGAAAGGCCGAA AGCUGGU
2185	CUCAAUA CUGAUGAGGCCGAAAGGCCGAA AUAGCUG
2186	ACUAAAU CUGAUGAGGCCGAAAGGCCGAA AAUAGCU
2187	CACUCAA CUGAUGAGGCCGAAAGGCCGAA AAAUACC
2189	GACACUC CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
2196	CAUAAA CUGAUGAGGCCGAAAGGCCGAA ACACUCA
2198	UACAUAA CUGAUGAGGCCGAAAGGCCGAA AGACACU
2199	CUACAU CUGAUGAGGCCGAAAGGCCGAA AAGACAC
2200	CCUACAU CUGAUGAGGCCGAAAGGCCGAA AAAGACA
2201	GGCUACA CUGAUGAGGCCGAAAGGCCGAA AAAAGAC
2205	UUUAGCC CUGAUGAGGCCGAAAGGCCGAA ACUAAA
2210	GUUCAUU CUGAUGAGGCCGAAAGGCCGAA AGCCUAC
2220	AGAGACC CUGAUGAGGCCGAAAGGCCGAA AUGUUCA
2224	GGCCAGA CUGAUGAGGCCGAAAGGCCGAA ACCUUAUG
2226	GAGGCCA CUGAUGAGGCCGAAAGGCCGAA AGACCUA
2233	GCUCOGU CUGAUGAGGCCGAAAGGCCGAA AGGCCAG
2242	GGACUGG CUGAUGAGGCCGAAAGGCCGAA AGCUCCG

2248 UGACAUG CUGAUGAGGCCGAAAGGCCGAA ACUGGG
 2254 UGAUUGU CUGAUGAGGCCGAAAGGCCGAA ACAUGGA
 2259 GACCUUG CUGAUGAGGCCGAAAGGCCGAA AUGUGAC
 2260 UGACCUU CUGAUGAGGCCGAAAGGCCGAA AAUGUGA
 2266 ACCUGGU CUGAUGAGGCCGAAAGGCCGAA ACCUUGA
 2274 ACAACUG CUGAUGAGGCCGAAAGGCCGAA ACCUGGU
 2279 CCUGUAC CUGAUGAGGCCGAAAGGCCGAA ACUGUAC
 2282 CAACCUUG CUGAUGAGGCCGAAAGGCCGAA ACAACTUG
 2288 AGUGUAC CUGAUGAGGCCGAAAGGCCGAA ACCUGUA
 2291 UGCAGUG CUGAUGAGGCCGAAAGGCCGAA ACAACCU
 2321 CCCAUUU CUGAUGAGGCCGAAAGGCCGAA AUCUUUU
 2338 CAAUGAG CUGAUGAGGCCGAAAGGCCGAA AGUCCCA
 2339 CCAAUGA CUGAUGAGGCCGAAAGGCCGAA AAGUCCC
 2341 GGCCAAU CUGAUGAGGCCGAAAGGCCGAA AGAAGUC
 2344 GUUGGCC CUGAUGAGGCCGAAAGGCCGAA AUGAGAA
 2358 CUGGGGA CUGAUGAGGCCGAAAGGCCGAA AGGCAGG
 2359 UCUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGGCAG
 2360 UUCUGGG CUGAUGAGGCCGAAAGGCCGAA AAAGGCA
 2376 AUAGAAA CUGAUGAGGCCGAAAGGCCGAA AUCACUC
 2377 GAUAGAA CUGAUGAGGCCGAAAGGCCGAA AAUCACU
 2378 CGAUAGA CUGAUGAGGCCGAAAGGCCGAA AAAUCAC
 2379 COGAUAG CUGAUGAGGCCGAAAGGCCGAA AAAAUCA
 2380 GCGGAUA CUGAUGAGGCCGAAAGGCCGAA AAAAAUC
 2382 GUGCCGA CUGAUGAGGCCGAAAGGCCGAA AGAAAAA
 2384 UUGUGCC CUGAUGAGGCCGAAAGGCCGAA AUAGAAA
 2399 GUCCAU A CUGAUGAGGCCGAAAGGCCGAA AGUGC
 2401 CAGUCCA CUGAUGAGGCCGAAAGGCCGAA AUAGUGC
 2411 GAACCAU CUGAUGAGGCCGAAAGGCCGAA ACCAGUC
 2417 ACCUGUG CUGAUGAGGCCGAAAGGCCGAA ACCAUUA
 2418 AACCUUG CUGAUGAGGCCGAAAGGCCGAA AACCAUU
 2425 AUCUCUG CUGAUGAGGCCGAAAGGCCGAA ACCUGUG
 2426 AAUCUCU CUGAUGAGGCCGAAAGGCCGAA AACCUGU
 2433 ACUGGGU CUGAUGAGGCCGAAAGGCCGAA AUCUCUG
 2434 CACUGGG CUGAUGAGGCCGAAAGGCCGAA AAUCUCU
 2448 GAGGAU CUGAUGAGGCCGAAAGGCCGAA AGGCCUC
 2449 GGAGGA CUGAUGAGGCCGAAAGGCCGAA AAGGCCU
 2451 AGGGAGG CUGAUGAGGCCGAAAGGCCGAA AUAAGGC
 2452 AAGGGAG CUGAUGAGGCCGAAAGGCCGAA AAUAAGG
 2455 GGGAAAG CUGAUGAGGCCGAAAGGCCGAA AGGAAUA
 2459 UGGGGGG CUGAUGAGGCCGAAAGGCCGAA AGGGAGG
 2460 UGGGGGG CUGAUGAGGCCGAAAGGCCGAA AAGGGAG
 2479 GCUAAC A CUGAUGAGGCCGAAAGGCCGAA AGGUGUC
 2480 GGCUCUAC CUGAUGAGGCCGAAAGGCCGAA AAGGUGU
 2483 GGUGGU CUGAUGAGGCCGAAAGGCCGAA ACAAAGG
 2484 AGGUGGC CUGAUGAGGCCGAAAGGCCGAA AACAAAG
 2492 GGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUGGC
 2504 AGAAAUG CUGAUGAGGCCGAAAGGCCGAA AUGUGGG
 2508 UGGCAGA CUGAUGAGGCCGAAAGGCCGAA AUGUAUG
 2509 CUGGCA CUGAUGAGGCCGAAAGGCCGAA AAUGUAU

2510 ACUGGCA CUGAUGAGGCCGAAAGGCCGAA AAADGUA
 2520 CAUUGUG CUGAUGAGGCCGAAAGGCCGAA ACACUGG
 2521 UCAJUGU CUGAUGAGGCCGAAAGGCCGAA AACACUG
 2533 GACCGCU CUGAUGAGGCCGAAAGGCCGAA AGUGUCA
 2540 CAGACAU CUGAUGAGGCCGAAAGGCCGAA ACGCTUG
 2545 AUGUCCA CUGAUGAGGCCGAAAGGCCGAA ACAUGAC
 2568 UUGGGCA CUGAUGAGGCCGAAAGGCCGAA AUUCCU
 2579 CAAGGCA CUGAUGAGGCCGAAAGGCCGAA AGCJUGG
 2585 AGAGGAC CUGAUGAGGCCGAAAGGCCGAA AGGCATA
 2588 ACAAGAG CUGAUGAGGCCGAAAGGCCGAA ACAAGGC
 2591 AGEACAA CUGAUGAGGCCGAAAGGCCGAA AGGACAA
 2593 ACAGGAC CUGAUGAGGCCGAAAGGCCGAA AGAGGAC
 2596 CAAACAG CUGAUGAGGCCGAAAGGCCGAA ACAAGAG
 2601 AAAUGCA CUGAUGAGGCCGAAAGGCCGAA ACAGGAC
 2602 GAAAUGC CUGAUGAGGCCGAAAGGCCGAA AACAGGA
 2607 CCAGUGA CUGAUGAGGCCGAAAGGCCGAA AUGCAA
 2608 CCCAGUG CUGAUGAGGCCGAAAGGCCGAA AADGCAA
 2609 UCCCAGU CUGAUCAGGCCGAAAGGCCGAA AAAUGCA
 2620 AUAGUGC CUGAUGAGGCCGAAAGGCCGAA AGCUOCC
 2626 GCUGCAA CUGAUGAGGCCGAAAGGCCGAA AGUGCAA
 2628 GAGCUGC CUGAUGAGGCCGAAAGGCCGAA AUAGUGC
 2635 GAAACUG CUGAUGAGGCCGAAAGGCCGAA AGCUGCA
 2640 UGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACUGGAG
 2641 CUGCAGG CUGAUGAGGCCGAAAGGCCGAA AACUGGA
 2642 ACUGCA G CUGAUGAGGCCGAAAGGCCGAA AAACUGG
 2653 GGACCCU CUGAUGAGGCCGAAAGGCCGAA AUCACUG
 2659 CUUUCAG CUGAUGAGGCCGAAAGGCCGAA ACCCUGA
 2689 CCUCCAA CUGAUGAGGCCGAAAGGCCGAA ACCUUGG
 2691 GUCCUCC CUGAUGAGGCCGAAAGGCCGAA AUACCUU
 2700 UGGGAGG CUGAUGAGGCCGAAAGGCCGAA AGUCCUC
 2704 AAGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGGAGU
 2711 CCUUCCA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
 2712 CCCUUCC CUGAUGAGGCCGAAAGGCCGAA AAGCUGG
 2721 CGCGGAU CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
 2724 ACACCGG CUGAUGAGGCCGAAAGGCCGAA AUGACCC
 2744 CUACACA CUGAUGAGGCCGAAAGGCCGAA ACACACA
 2750 GCUUGUC CUGAUGAGGCCGAAAGGCCGAA ACACATA
 2759 AGAGCGA CUGAUGAGGCCGAAAGGCCGAA AGCUUGU
 2761 ACAGAGC CUGAUGAGGCCGAAAGGCCGAA AGAGCUU
 2765 GGUGACA CUGAUGAGGCCGAAAGGCCGAA AGCGAGA
 2769 CCUGGGU CUGAUGAGGCCGAAAGGCCGAA ACAGAGC
 2797 GAACCAU CUGAUGAGGCCGAAAGGCCGAA AUUGCAC
 2803 UGCAGUG CUGAUGAGGCCGAAAGGCCGAA ACCAUGA
 2804 CUGCAGU CUGAUGAGGCCGAAAGGCCGAA AACCAG
 2813 AGGUCAA CUGAUGAGGCCGAAAGGCCGAA ACUGCAG
 2815 AAAGGUC CUGAUGAGGCCGAAAGGCCGAA AGACUGC
 2821 AGCCCCA CUGAUGAGGCCGAAAGGCCGAA AGGUCAA
 2822 GAGCCCC CUGAUGAGGCCGAAAGGCCGAA AAGGUCA
 2823 UAGAGCC CUGAUGAGGCCGAAAGGCCGAA AAAGGUC

2829	AUCACUU CUGAUGAGGCCGAAAGGCCGAA AGCCCAA
2837	GUGGGAG CUGAUGAGGCCGAAAGGCCGAA AUCACUU
2840	GAGGUGG CUGAUGAGGCCGAAAGGCCGAA AGGAUCA
2847	GGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUGGG
2853	UACUCAG CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
2860	UCCCAGC CUGAUGAGGCCGAAAGGCCGAA ACUCAGG
2872	GUGAGCC CUGAUGAGGCCGAAAGGCCGAA AUGGUCC
2877	GUGUJGU CUGAUGAGGCCGAAAGGCCGAA AGCCUAU
2899	AAAAUCA CUGAUGAGGCCGAAAGGCCGAA AUUUGCC
2900	AAAAAUU CUGAUGAGGCCGAAAGGCCGAA AAUUUGC
2904	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AUCAAAU
2905	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAUCAAA
2906	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAUCAA
2907	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAUCA
2908	AAAAAAA CUGADGAGGCCGAAAGGCCGAA AAAAUJC
2909	AAAAAAA CUGADGAGGCCGAAAGGCCGAA AAAAUAU
2910	AAAAAAA CUGADGAGGCCGAAAGGCCGAA AAAAUAAA
2911	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAUAAA
2912	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAUAAA
2913	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAUAAA
2914	AAAAAAA CUGAUGAGGCCGAAAGGCCGAA AAAAUAAA
2915	UCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAAUAAA
2916	CUCUGAA CUGAUGAGGCCGAAAGGCCGAA AAAAUAAA
2917	UCUCUGA CUGAUGAGGCCGAAAGGCCGAA AAAAUAAA
2918	GUCUCUG CUGAUGAGGCCGAAAGGCCGAA AAAAUAAA
2919	CGUCUCU CUGAUGAGGCCGAAAGGCCGAA AAAAUAAA
2931	GUUGCGA CUGAUGAGGCCGAAAGGCCGAA ACCCGU
2933	AUGUUGC CUGAUGAGGCCGAAAGGCCGAA AGACCCC
2941	UCUJGGC CUGAUGAGGCCGAAAGGCCGAA AUGUUGC
2951	ACAAAGG CUGAUGAGGCCGAAAGGCCGAA AGUCUGG
2952	CACAAAG CUGAUGAGGCCGAAAGGCCGAA AAGUCUG
2955	UAACACA CUGAUGAGGCCGAAAGGCCGAA AGGAAGU
2956	CUAACAC CUGAUGAGGCCGAAAGGCCGAA AAGGAAG
2961	AUUAACU CUGAUGAGGCCGAAAGGCCGAA ACACAAA
2962	UAUUAAC CUGAUGAGGCCGAAAGGCCGAA AACACAA
2965	CUUUUU CUGAUGAGGCCGAAAGGCCGAA ACTAACAC
2966	GCUUUUAU CUGAUGAGGCCGAAAGGCCGAA AACUAAAC
2969	AAAGCUU CUGAUGAGGCCGAAAGGCCGAA AUUAACU
2975	GUUGAGA CUGAUGAGGCCGAAAGGCCGAA AGCUUUUA
2976	AGUUGAG CUGAUGAGGCCGAAAGGCCGAA AAGCUUU
2977	CAGUUGA CUGAUGAGGCCGAAAGGCCGAA AAAGCUU
2979	GGCAGUU CUGAUGAGGCCGAAAGGCCGAA AGAAAGC

Table 5

Mouse ICAM HH Ribozyme Sequence
nt. Position Ribozyme Sequence

11 CAAAGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
23 AGCAGAG CUGAUGAGGCCGAAAGGCCGAA ACCACUG
26 AGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGAACCC
31 UGUGGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
34 CGACCCU CUGAUGAGGCCGAAAGGCCGAA AUGAGAA
40 AGGCUAC CUGAUGAGGCCGAAAGGCCGAA AGUGUGC
48 CCAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
54 CCAUCAC CUGAUGAGGCCGAAAGGCCGAA AGGCCCA
58 GGAGCUA CUGAUGAGGCCGAAAGGCCGAA AGGCAUG
64 CUGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG
96 GGGCCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGAG
102 CCAGCAG CUGAUGAGGCCGAAAGGCCGAA ACUGGCA
108 GGGCCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
115 AGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGAACCA
119 UCCUGGU CUGAUGAGGCCGAAAGGCCGAA ACAUCCC
120 GGGCCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
146 GGAAGCG CUGAUGAGGCCGAAAGGCCGAA ACGACUG
152 AGUGGCU CUGAUGAGGCCGAAAGGCCGAA ACACAGA
158 GGUUUUU CUGAUGAGGCCGAAAGGCCGAA AACAGGA
165 GCAAAAC CUGAUGAGGCCGAAAGGCCGAA ACUUCUG
168 GGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUU
185 CUGCACG CUGAUGAGGCCGAAAGGCCGAA ACCCACC
209 GCCAGAG CUGAUGAGGCCGAAAGGCCGAA AAGUGGC
227 GCAAAAC CUGAUGAGGCCGAAAGGCCGAA ACUUCUG
230 GGAGCAA CUGAUGAGGCCGAAAGGCCGAA ACAACUU
237 AGUUCUC CUGAUGAGGCCGAAAGGCCGAA AACACAG
248 UUUAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGGUU
253 UCUUCCU CUGAUGAGGCCGAAAGGCCGAA AGGCAGG
263 CAGUAGA CUGAUGAGGCCGAAAGGCCGAA AAAACCU
267 UAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCCCCU
293 CAGCUCA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
319 GGCUCAG CUGAUGAGGCCGAAAGGCCGAA AUCUCUU
335 GUUCUCA CUGAUGAGGCCGAAAGGCCGAA AGCACAG
337 CAGUGUG CUGAUGAGGCCGAAAGGCCGAA AUUGGAC
338 UCAGCUC CUGAUGAGGCCGAAAGGCCGAA AACAGCU
359 AGCGGAC CUGAUGAGGCCGAAAGGCCGAA ACUGCAC
367 CGGGUUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
374 GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGCCUUC
375 GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUU
378 ACACGGU CUGAUGAGGCCGAAAGGCCGAA AUGGUAG
386 AAACGAA CUGAUGAGGCCGAAAGGCCGAA ACACGGU
394 AGAUUGA CUGAUGAGGCCGAAAGGCCGAA AGUCCGG
20 CGGGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUGUG
25 CUGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG

427 CACUGGU CUGAUGAGGCCGAAAGGCCGAA AGAGCUG
 450 GCAGGGU CUGAUJGAGGCCGAAAGGCCGAA AGGUCCU
 451 CAAAGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUC
 456 AGUGGCCU CUGAUGAGGCCGAAAGGCCGAA AGGGUAA
 495 ACACGGU CUGAUGAGGCCGAAAGGCCGAA AUGGUAG
 510 CCCACCG CUGAUGAGGCCGAAAGGCCGAA ACCAGCA
 564 GGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACCUGAG
 592 CCCAUGU CUGAUGAGGCCGAAAGGCCGAA AUUUUC
 607 CAUGACA CUGAUGAGGCCGAAAGGCCGAA AUUGGCU
 608 GCAUGAG CUGAUGAGGCCGAAAGGCCGAA AAUUGC
 609 GGGCAU CUGAUGAGGCCGAAAGGCCGAA AAAUUGG
 611 CAGCUCA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
 656 657 UCAGCUC CUGAUGAGGCCGAAAGGCCGAA AACAGCU
 668 GGUGGCC CUGAUGAGGCCGAAAGGCCGAA AGGCUCG
 677 AGGCUGG CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
 684 AGGACCG CUGAUGAGGCCGAAAGGCCGAA ACCUGAA
 692 AAGAUCG CUGAUGAGGCCGAAAGGCCGAA AAGUCCG
 693 GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
 696 GAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGG
 709 UGAGGUG CUGAUGAGGCCGAAAGGCCGAA AGCCGCC
 720 AGCUGAA CUGAUGAGGCCGAAAGGCCGAA AGUUGUA
 723 CGGAGCU CUGAUCAGGCCGAAAGGCCGAA AAAAGUU
 735 UCUCCAAG CUGAUGAGGCCGAAAGGCCGAA AUCUGGU
 738 CCAUCAC CUGAUGAGGCCGAAAGGCCGAA AGGCCCA
 765 GGAAGCG CUGAUGAGGCCGAAAGGCCGAA ACGACUG
 769 GGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
 770 UUCCAGG CUGAUGAGGCCGAAAGGCCGAA AGCAAAA
 785 GGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
 786 AGGCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGGC
 792 CUUCCGA CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
 794 AGUCUCC CUGAUGAGGCCGAAAGGCCGAA AGCCCAG
 807 CCAGGUA CUGAUGAGGCCGAAAGGCCGAA AUCCGAG
 833 GEGUGUC CUGAUGAGGCCGAAAGGCCGAA AGCUUUG
 846 CAACGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
 851 GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AGGUCUC
 863 CCAGAGG CUGAUGAGGCCGAAAGGCCGAA AGUGGU
 866 GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGUUC
 867 UCUCCGG CUGAUGAGGCCGAAAGGCCGAA AACGAU
 869 CUUCAU CUGAUGAGGCCGAAAGGCCGAA AGGAAGA
 881 ACGGGUU CUGAUGAGGCCGAAAGGCCGAA AAGCCAU
 885 UCACUC CUGAUGAGGCCGAAAGGCCGAA ACCAAGG
 933 CCAGAAU CUGAUGAGGCCGAAAGGCCGAA AUUAUAG
 936 GCACCAAG CUGAUGAGGCCGAAAGGCCGAA AUGAUUA
 978 AGUUGUA CUGAUGAGGCCGAAAGGCCGAA ACUGUUA
 980 AAAGUUG CUGAUGAGGCCGAAAGGCCGAA AGACUGU
 986 AGCUGAA CUGAUGAGGCCGAAAGGCCGAA AGUUGUA
 987 GGGCUGA CUGAUGAGGCCGAAAGGCCGAA AAGUUGU
 988 GGAGCTG CUGAUGAGGCCGAAAGGCCGAA AAA.GUUG

1005 UCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUCUGGU
 1006 UUCCCCA CUGAUGAGGCCGAAAGGCCGAA ACUCUCA
 1023 CUUCCGA CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
 1025 CCCUUCC CUGAUGAGGCCGAAAGGCCGAA AGACCUC
 1066 UUAUUUU CUGAUGAGGCCGAAAGGCCGAA AGAGUGG
 1092 GGCCUGA CUGAUGAGGCCGAAAGGCCGAA AUCCAGU
 1093 UGGCUG CUGAUGAGGCCGAAAGGCCGAA AGGUCCA
 1125 UCAAGAA CUGAUGAGGCCGAAAGGCCGAA AGUUGGG
 1163 GCAAAAG CUGAUGAGGCCGAAAGGCCGAA AGCUUCG
 1164 AGCAAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUC
 1166 AGAGCAA CUGAUGAGGCCGAAAGGCCGAA AGAACGU
 1172 GGUUUUU CUGAUGAGGCCGAAAGGCCGAA AACAGGA
 1200 UGUGGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
 1201 CUGUUCA CUGAUGAGGCCGAAAGGCCGAA AAGCAGC
 1203 ACUGGUG CUGAUGAGGCCGAAAGGCCGAA AAAAAGU
 1227 GCACACG CUGAUGAGGCCGAAAGGCCGAA AUGUACC
 1228 AGCAAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUC
 1233 CUUCUCG CUGAUGAGGCCGAAAGGCCGAA AAACGAA
 1238 AGGACCA CUGAUGAGGCCGAAAGGCCGAA ACAGCAC
 1264 CUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
 1267 UUCCCCA CUGAUGAGGCCGAAAGGCCGAA ACUCUCA
 1294 GGCUCAG CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
 1295 CUGCGUA CUGAUGAGGCCGAAAGGCCGAA ACCCCCUC
 1306 CAUUUCA CUGAUGAGGCCGAAAGGCCGAA AGUCUGC
 1321 UCCUCCU CUGAUGAGGCCGAAAGGCCGAA AGCCUUC
 1334 UUUAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGGUU
 1344 CACUCUC CUGAUGAGGCCGAAAGGCCGAA AGCUCAU
 1351 UAACUUA CUGAUGAGGCCGAAAGGCCGAA ACAUUCA
 1353 CACCUUC CUGAUGAGGCCGAAAGGCCGAA ACCCACU
 1366 AGUUGUA CUGAUGAGGCCGAAAGGCCGAA ACUGUUA
 1367 AGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUGCU
 1368 AGAGUGG CUGAUGAGGCCGAAAGGCCGAA ACAGUAC
 1380 CCACCCC CUGAUGAGGCCGAAAGGCCGAA AUGGGCA
 1388 AGCCACU CUGAUGAGGCCGAAAGGCCGAA AGUCUCC
 1398 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAGCCA
 1402 AGUUCUC CUGAUGAGGCCGAAAGGCCGAA AAGCACA
 1408 CCUCCCC CUGAUGAGGCCGAAAGGCCGAA AUCUOGC
 1410 CCCUUCC CUGAUGAGGCCGAAAGGCCGAA AGACCUC
 1421 ACAAAAG CUGAUGAGGCCGAAAGGCCGAA AGGUGGG
 1425 CUCUACC CUGAUGAGGCCGAAAGGCCGAA AGGCAGU
 1429 CAGGGGC CUGAUGAGGCCGAAAGGCCGAA AUAGAGA
 1444 UCCUCCU CUGAUGAGGCCGAAAGGCCGAA AGCCUUC
 1455 UCCUGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUCC
 1482 GGGAGCA CUGAUGAGGCCGAAAGGCCGAA AACAAACU
 1484 CAUGAGG CUGAUGAGGCCGAAAGGCCGAA AGAACAG
 1493 GUUCUCA CUGAUGAGGCCGAAAGGCCGAA AGCACAG
 1500 GGACCAU CUGAUGAGGCCGAAAGGCCGAA AUUCAU
 1503 GAAUGAU CUGAUGAGGCCGAAAGGCCGAA AUAGUCC
 1506 CGGUUAU CUGAUGAGGCCGAAAGGCCGAA AACAUAA

1509 ACACGGU CUGAUGAGGCCGAAAGGCCGAA AUGGUAG
 1518 CGCCUGG CUGAUGAGGCCGAAAGGCCGAA ACCAUGA
 1530 CCAGAAU CUGAUGAGGCCGAAAGGCCGAA AUUAUAG
 1533 GCCCCAC CUGAUGAGGCCGAAAGGCCGAA AUGACCA
 1551 ACCUGCU CUGAUGAGGCCGAAAGGCCGAA AGGC AUG
 1559 AGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUGCU
 1563 GGUUAUA CUGAUGAGGCCGAAAGGCCGAA ACUUAAG
 1565 GCGGUUA CUGAUGAGGCCGAAAGGCCGAA AAACAU
 1567 UGGCGGU CUGAUGAGGCCGAAAGGCCGAA AUAAAACA
 1584 AUAUCCU CUGAUGAGGCCGAAAGGCCGAA AUCUUUC
 1592 UAACUUG CUGAUGAGGCCGAAAGGCCGAA AUAUCCU
 1599 CCUUCUG CUGAUGAGGCCGAAAGGCCGAA AACUUGU
 1651 GCUCAGG CUGAUGAGGCCGAAAGGCCGAA AGGUGGG
 1661 CAAAGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUUC
 1663 UUCAAAG CUGAUGAGGCCGAAAGGCCGAA AAAGGUU
 1678 CCAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
 1680 CCAGAGG CUGAUGAGGCCGAAAGGCCGAA AGUGGGU
 1681 GCCAGAG CUGAUGAGGCCGAAAGGCCGAA AAUGUGG
 1684 ACAGCCA CUGAUGAGGCCGAAAGGCCGAA AGGAAGU
 1690 AGAUUCG CUGAUGAGGCCGAAAGGCCGAA AGUCCGG
 1691 AAGAUCCG CUGAUGAGGCCGAAAGGCCGAA AAGUCCG
 1696 CCACCCC CUGAUGAGGCCGAAAGGCCGAA AUGGGCA
 1698 CUCCAGG CUGAUGAGGCCGAAAGGCCGAA AUAUCCG
 1737 GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AGGUUC
 1750 UGAGGUG CUGAUGAGGCCGAAAGGCCGAA AGCCGCC
 1756 GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGUUC
 1787 UGGGGAC CUGAUGAGGCCGAAAGGCCGAA AUGUCUC
 1790 AUUAGAG CUGAUGAGGCCGAAAGGCCGAA ACAAUGC
 1793 UCCAGCC CUGAUGAGGCCGAAAGGCCGAA AGGACCA
 1797 UUUAGU CUGAUGAGGCCGAAAGGCCGAA ACUGGUG
 1802 UCUCAG CUGAUGAGGCCGAAAGGCCGAA AUCUGGU
 1812 GGCCUGA CUGAUGAGGCCGAAAGGCCGAA AUCCAGU
 1813 UGAGGGU CUGAUGAGGCCGAAAGGCCGAA AAUGCUG
 1825 GCAGAGG CUGAUGAGGCCGAAAGGCCGAA AGOGUGG
 1837 GGAGCUA CUGAUGAGGCCGAAAGGCCGAA AGGCAUG
 1845 GGUGGCC CUGAUGAGGCCGAAAGGCCGAA AGGCCUC
 1856 AAGAUCCG CUGAUGAGGCCGAAAGGCCGAA AAGUCCG
 1861 UACUGGA CUGAUGAGGCCGAAAGGCCGAA AUCAUGU
 1865 CUGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAAGUG
 1868 UUUAGU CUGAUGAGGCCGAAAGGCCGAA ACUGGUG
 1877 AGCUGCU CUGAUGAGGCCGAAAGGCCGAA AGGCAUG
 1901 GUCCCCU CUGAUGAGGCCGAAAGGCCGAA AGUUUUUA
 1912 ACUGAAC CUGAUGAGGCCGAAAGGCCGAA ACUUAU
 1922 UAACUUA CUGAUGAGGCCGAAAGGCCGAA ACAUUC
 1923 GAUACCU CUGAUGAGGCCGAAAGGCCGAA AGCAUCA
 1928 CUGGUUA CUGAUGAGGCCGAAAGGCCGAA ACUCUAA
 1930 AGCUGGU CUGAUGAGGCCGAAAGGCCGAA AAACUC
 1964 UGGGGAC CUGAUGAGGCCGAAAGGCCGAA AUGUCUC
 1983 UAACUUG CUGAUGAGGCCGAAAGGCCGAA AUAUCCU

1996	GGCUCAG CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
2005	GGUCCGC CUGAUGAGGCCGAAAGGCCGAA AGCUCCA
2013	UACUCAA CUGAUGAGGCCGAAAGGCCGAA AAAUAGC
2015	CCACCCC CUGAUGAGGCCGAAAGGCCGAA AUGGGCA
2020	CUCAGAA CUGAUGAGGCCGAAAGGCCGAA AACCAAC
2039	CCUCUGC CUGAUGAGGCCGAAAGGCCGAA AGCCAGC
2040	CCUCCAG CUGAUGAGGCCGAAAGGCCGAA AGGUCAAG
2057	GGAUUGUG CUGAUGAGGCCGAAAGGCCGAA AGGAGCA
2061	ACACGGU CUGAUGAGGCCGAAAGGCCGAA AUGGUAG
2071	CUGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAAGUJG
2076	UAGCUCU CUGAUGAGGCCGAAAGGCCGAA AGGCUAC
2097	CAUCAAG CUGAUGAGGCCGAAAGGCCGAA AGAGJUG
2098	CGGGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUGUG
2115	AUCCUCC CUGAUGAGGCCGAAAGGCCGAA AGCUGGC
2128	CUCAAAU CUGAUGAGGCCGAAAGGCCGAA AUAGCUG
2130	GAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGG
2145	CAUCAAG CUGAUGAGGCCGAAAGGCCGAA AGAGUJG
2152	AACUCUA CUGAUGAGGCCGAAAGGCCGAA AUUAAAUA
2156	UUUAAA CUGAUGAGGCCGAAAGGCCGAA ACAUCAA
2158	UUUAAA CUGAUGAGGCCGAAAGGCCGAA AUACAU
2159	UUUAAA CUGAUGAGGCCGAAAGGCCGAA AAUACAU
2160	AAAUAUA CUGAUGAGGCCGAAAGGCCGAA AAAUACA
2162	CUAAAUU CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
2163	AAUUAUA CUGAUGAGGCCGAAAGGCCGAA AAUACAU
2166	AAUAGAG CUGAUGAGGCCGAAAGGCCGAA AUGAAGU
2167	AAUUAUA CUGAUGAGGCCGAAAGGCCGAA AAUACAU
2170	CUAAAUU CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
2171	GGGAGCA CUGAUGAGGCCGAAAGGCCGAA AACAAACU
2173	CUUGUAA CUGAUGAGGCCGAAAGGCCGAA ACUCUAA
2174	GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AACUCUA
2175	AGCUGGU CUGAUGAGGCCGAAAGGCCGAA AAACUCU
2176	UAGCUGG CUGAUGAGGCCGAAAGGCCGAA AAAACUC
2183	CAAUAAA CUGAUGAGGCCGAAAGGCCGAA AGCUGGU
2185	CUAAAU CUGAUGAGGCCGAAAGGCCGAA AUAGCUG
2186	ACUCAAU CUGAUGAGGCCGAAAGGCCGAA AAUAGCU
2187	UACUCAA CUGAUGAGGCCGAAAGGCCGAA AAAUAGC
2189	GGUACUC CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
2196	CAUCAAG CUGAUGAGGCCGAAAGGCCGAA AGAGUUG
2198	AAACUAA CUGAUGAGGCCGAAAGGCCGAA AGGCUGC
2199	AAACUAA CUGAUGAGGCCGAAAGGCCGAA AAGAGGC
2200	CUUGCAU CUGAUGAGGCCGAAAGGCCGAA AGGAAGA
2201	GCCGACA CUGAUGAGGCCGAAAGGCCGAA AAAACUU
2205	UCAGGCC CUGAUGAGGCCGAAAGGCCGAA ACAUAAA
2210	AGCCACU CUGAUGAGGCCGAAAGGCCGAA AGUCUCC
2220	AGAGAAC CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
2224	GGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACCUGAG
2226	GCGGCCU CUGAUGAGGCCGAAAGGCCGAA AGAUCCA
2233	CCUCCAG CUGAUGAGGCCGAAAGGCCGAA AGGUCAAG
2242	GGUCCGC CUGAUGAGGCCGAAAGGCCGAA AGCUCCA

2248 UGGGAUG CUGAUGAGGCCGAAAGGCCGAA AUGGAUA
2254 UCAGUGU CUGAUGAGGCCGAAAGGCCGAA AAUUGGA
2259 CACCGUG CUGAUGAGGCCGAAAGGCCGAA AUGUGAU
2260 GCACCGU CUGAUGAGGCCGAAAGGCCGAA AAUGUGA
2266 UCCUGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUC
2274 UCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUCUGGU
2279 CUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
2282 CAGCUCA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
2288 AGGCCAU CUGAUGAGGCCGAAAGGCCGAA ACUUAUA
2291 AGCAGAG CUGAUGAGGCCGAAAGGCCGAA ACCACUG
2321 CCCAUGU CUGAUGAGGCCGAAAGGCCGAA AUCUUUC
2338 CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGUCUCA
2339 CAAAGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUUC
2341 AGGCUGG CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
2344 GCUGGAA CUGAUGAGGCCGAAAGGCCGAA AUCGAAA
2358 CUGCUCA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
2359 UCUGUUC CUGAUGAGGCCGAAAGGCCGAA AAAGCAG
2360 UUCAAAG CUGAUGAGGCCGAAAGGCCGAA AAAGGUU
2376 UCAGAAG CUGAUGAGGCCGAAAGGCCGAA ACCACCU
2377 CUCAGAA CUGAUGAGGCCGAAAGGCCGAA AACCAAC
2378 CAGUTAGA CUGAUGAGGCCGAAAGGCCGAA AAACCCU
2379 CUUAUGA CUGAUGAGGCCGAAAGGCCGAA AAAAGCA
2380 GCGGACA CUGAUGAGGCCGAAAGGCCGAA AAAACUU
2382 GGGGCAA CUGAUGAGGCCGAAAGGCCGAA AGAGAAU
2384 UUGUGUC CUGAUGAGGCCGAAAGGCCGAA ACUGGAU
2399 GUCCACA CUGAUGAGGCCGAAAGGCCGAA AGUGUUU
2401 CAGCUCA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
2411 GCAUCCU CUGAUGAGGCCGAAAGGCCGAA ACCAGUA
2417 ACGUAUG CUGAUGAGGCCGAAAGGCCGAA ACCAUUC
2418 GGCCUGA CUGAUGAGGCCGAAAGGCCGAA AUCCAGU
2425 AACCCUC CUGAUGAGGCCGAAAGGCCGAA ACCCAUG
2426 AAACUCU CUGAUGAGGCCGAAAGGCCGAA AAUUAU
2433 GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AACUCUA
2434 AGCUGGU CUGAUGAGGCCGAAAGGCCGAA AAACUCU
2448 GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCUUC
2449 GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUU
2451 AGGCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGGC
2452 GAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGG
2455 GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCUUC
2459 GGGGGGG CUGAUGAGGCCGAAAGGCCGAA AGUGUGG
2460 CGGGGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUGUG
2479 GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AGGUCUC
2480 GGAUCAC CUGAUGAGGCCGAAAGGCCGAA ACGGUGA
2483 GGUGGCU CUGAUGAGGCCGAAAGGCCGAA ACAUUGG
2484 GACUGGU CUGAUGAGGCCGAAAGGCCGAA AAAAAAG
2492 AGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUGGU
2504 ACAAAAG CUGAUGAGGCCGAAAGGCCGAA AGGUGGG
2508 UGGGAUG CUGAUGAGGCCGAAAGGCCGAA AUGGAUA
2509 CUGGUAA CUGAUGAGGCCGAAAGGCCGAA ACUCUAA

2510 GCUGGUUA CUGAUGAGGCCGAAAGGCCGAA AACUCUA
 2520 CAUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAAAAG
 2521 UGAGGGU CUGAUGAGGCCGAAAGGCCGAA AUUGCUG
 2533 GAUACCU CUGAUGAGGCCGAAAGGCCGAA AGCAUCA
 2540 CACAGCG CUGAUGAGGCCGAAAGGCCGAA ACUGCUG
 2545 AGGACCA CUGAUGAGGCCGAAAGGCCGAA ACAGCAC
 2568 UUJUGACA CUGAUGAGGCCGAAAGGCCGAA ACUUAC
 2579 CAGGCCA CUGAUGAGGCCGAAAGGCCGAA AACUUAU
 2585 AGAGAAC CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
 2588 AUUAGAG CUGAUGAGGCCGAAAGGCCGAA ACAADGC
 2591 AGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGAACCA
 2593 GCAGAGC CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
 2596 CAUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAAAAG
 2601 AAACGAA CUGAUGAGGCCGAAAGGCCGAA ACACGGU
 2602 GGGGAUGG CUGAUGAGGCCGAAAGGCCGAA ACCUGGA
 2607 CCAGGUA CUGAUGAGGCCGAAAGGCCGAA AUCCGAG
 2608 CACAGCG CUGAUGAGGCCGAAAGGCCGAA ACUGCUG
 2609 UCCUGGU CUGAUGAGGCCGAAAGGCCGAA ACUUCCC
 2620 GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
 2626 GCUGGAA CUGAUGAGGCCGAAAGGCCGAA AUCCGAA
 2628 AGGCUAC CUGAUGAGGCCGAAAGGCCGAA AGUGUGC
 2635 AGGACCG CUGAUGAGGCCGAAAGGCCGAA AGCUGAA
 2640 GGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
 2641 CUGCUGA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
 2642 GAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGG
 2653 GCAUCCU CUGAUGAGGCCGAAAGGCCGAA ACCAGUA
 2659 CUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
 2689 CCUCGGA CUGAUGAGGCCGAAAGGCCGAA ACAUUAG
 2691 GGCCUCG CUGAUGAGGCCGAAAGGCCGAA AGACAUU
 2700 GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGUUUC
 2704 AGGCUGG CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
 2711 CUGCUGA CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
 2712 CCCUUCG CUGAUGAGGCCGAAAGGCCGAA AGACCUC
 2721 CUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCCUUC
 2724 GCACACG CUGAUGAGGCCGAAAGGCCGAA AUGUACC
 2744 CUGCAGC CUGAUGAGGCCGAAAGGCCGAA ACCCACC
 2750 GGUACUC CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 2759 AGAUCGA CUGAUGAGGCCGAAAGGCCGAA AGUCCGG
 2761 GCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
 2765 AGGGGCA CUGAUGAGGCCGAAAGGCCGAA AGCAAAA
 2769 CCUGUUU CUGAUGAGGCCGAAAGGCCGAA ACAGACU
 2797 GGACCAU CUGAUGAGGCCGAAAGGCCGAA AUUUCAU
 2803 CGCCUGG CUGAUGAGGCCGAAAGGCCGAA ACCAUGA
 2804 CUGCACG CUGAUGAGGCCGAAAGGCCGAA ACCCACC
 2813 GGGUCAG CUGAUGAGGCCGAAAGGCCGAA ACCGGAG
 2815 AAAGUUG CUGAUGAGGCCGAAAGGCCGAA AGACUGU
 2821 CCUCCAG CUGAUGAGGCCGAAAGGCCGAA AGGUCCAG
 2822 AAGUCCG CUGAUGAGGCCGAAAGGCCGAA AGGUCCU
 2823 UGGGAGC CUGAUGAGGCCGAAAGGCCGAA AAAGGCCA

2829 AUGAUUA CUGAUGAGGCCGAAAGGCCGAA AGUCCAG
2837 UCAGAAG CUGAUGAGGCCGAAAGGCCGAA ACCACCU
2840 CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGUCUCA
2847 GGUGGCU CUGAUGAGGCCGAAAGGCCGAA ACAUUGG
2853 AACAUAA CUGAUGAGGCCGAAAGGCCGAA AGGCUGC
2860 UCACAGU CUGAUGAGGCCGAAAGGCCGAA ACUUGGC
2872 CUUGGCU CUGAUGAGGCCGAAAGGCCGAA AAGGUCC
2877 GUGAUGG CUGAUGAGGCCGAAAGGCCGAA AGCGGAA
2899 AAGAUCG CUGAUGAGGCCGAAAGGCCGAA AAGUCCG
2900 AAAACUC CUGAUGAGGCCGAAAGGCCGAA AAAUAAA
2904 AAUAGAG CUGAUGAGGCCGAAAGGCCGAA AUGAAGU
2905 CAAUAGA CUGAUGAGGCCGAAAGGCCGAA AAUGAAG
2906 UAAUAAA CUGAUGAGGCCGAAAGGCCGAA ACAUCAA
2907 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAAUACA
2908 AGCAAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUC
2909 AGAGCAA CUGAUGAGGCCGAAAGGCCGAA AGAACGU
2910 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAAUACA
2911 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAAUACA
2912 GACAUUA CUGAUGAGGCCGAAAGGCCGAA AGAACAA
2913 UGACCAG CUGAUGAGGCCGAAAGGCCGAA AGAGAAA
2914 CUUAUGA CUGAUGAGGCCGAAAGGCCGAA AAAAGCA
2915 UCUAAAU CUGAUGAGGCCGAAAGGCCGAA AAUAAA
2916 CUCCGGA CUGAUGAGGCCGAAAGGCCGAA ACGAAUA
2917 UCUCGG CUGAUGAGGCCGAAAGGCCGAA AACGAAU
2918 CUCUCCG CUGAUGAGGCCGAAAGGCCGAA AAACGAA
2919 CGACCCU CUGAUGAGGCCGAAAGGCCGAA AUGAGAA
2931 CUUCCGA CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
2933 CCCUUCC CUGAUGAGGCCGAAAGGCCGAA AGACCU
2941 UGGGGAC CUGAUGAGGCCGAAAGGCCGAA AUGUCUC
2951 GCAGAGG CUGAUGAGGCCGAAAGGCCGAA AGCGUGG
2952 CACAGCG CUGAUGAGGCCGAAAGGCCGAA ACUGCUG
2955 UGACACA CUGAUGAGGCCGAAAGGCCGAA AGUCACU
2956 UUGAUUC CUGAUGAGGCCGAAAGGCCGAA AAGGAAA
2961 AGUGGCU CUGAUGAGGCCGAAAGGCCGAA ACACAGA
2962 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUACAU
2965 CUUUAUU CUGAUGAGGCCGAAAGGCCGAA AUUCAAA
2966 CCUCUGC CUGAUGAGGCCGAAAGGCCGAA AGCCAGC
2969 AAAACUU CUGAUGAGGCCGAAAGGCCGAA AUUGAUU
2975 GCUGGUA CUGAUGAGGCCGAAAGGCCGAA AACUCUA
2976 AGUAGAG CUGAUGAGGCCGAAAGGCCGAA AACCCUC
2977 CAGCUCA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
2979 GGCAAUA CUGAUGAGGCCGAAAGGCCGAA AGAAUGA

Table 6
Human ICAM Hairpin Ribozyme/Substrate Sequences
Hairpin Ribozyme Sequence

Position	nt.	Substrate
70		GAGCA CCCGGGCC
86		GGGGGG AGAA GCUG ACCAGAGAACACAGUUCGUUACAUUCCUGUA
343		GGGC ACCAGAGAACACAGUUCGUUACAUUCCUGUA
635		CCCNUCAG AGAA GUUU ACCAGAGAACACAGUUCGUUACAUUCCUGUA
653		GGCCUUGG AGAA GCAG ACCAGAGAACACAGUUCGUUACAUUCCUGUA
782		UGUUCUCA AGRA GCUC ACCAGAGAACACAGUUCGUUACAUUCCUGUA
920		AGACUGGG AGAA GCCC ACCAGAGAACACAGUUCGUUACAUUCCUGUA
1301		CUGCACAC AGAA GCGG ACCAGAGAACACAGUUCGUUACAUUCCUGUA
1373		ACAUUUGG AGAA GCUG ACCAGAGAACACAGUUCGUUACAUUCCUGUA
1521		CCCCGAUG AGAA GUGG ACCAGAGAACACAGUUCGUUACAUUCCUGUA
1594		AUGACUGC AGAA GCUA ACCAGAGAACACAGUUCGUUACAUUCCUGUA
2008		CUGUUGUA AGAA GURU ACCAGAGAACACAGUUCGUUACAUUCCUGUA
2034		ACCCAUA AGAA GCAA ACCAGAGAACACAGUUCGUUACAUUCCUGUA
2125		UDUCUGUAA AGAA GUGG ACCAGAGAACACAGUUCGUUACAUUCCUGUA
2132		GGUCAGUA AGAA CGAG ACCAGAGAACACAGUUCGUUACAUUCCUGUA
2276		GGGUUUGGG AGAA GUAG ACCAGAGAACACAGUUCGUUACAUUCCUGUA
2810		ACCUUGUC AGAA GUAC ACCAGAGAACACAGUUCGUUACAUUCCUGUA
		AAGGUCAA AGAA GGAG ACCAGAGAACACAGUUCGUUACAUUCCUGUA

Table 7
Mouse ICAM Hairpin Ribozyme/Substrate Sequences
Hairpin Ribozyme Sequence

nt. Position	Substrate
76	GGGAUCAC AGAA GUGA ACCAGAGAAACACGUGGUACAUUACCUUGUA
164	UGAGGGAG AGAA GUUC ACCAGAGAAACACGUGGUACAUUACCUUGUA
252	UCAGCCUCA AGAA GCUU ACCAGAGAAACACGUGGUACAUUACCUUGUA
284	GCACAGCG AGAA GCUG ACCAGAGAAACACGUGGUACAUUACCUUGUA
318	AAGCGGAC AGAA GCAC ACCAGAGAAACACGUGGUACAUUACCUUGUA
447	AGAGCUGG AGAA GCGG ACCAGAGAAACACGUGGUACAUUACCUUGUA
804	UCUCCUGG AGAA GCAU ACCAGAGAAACACGUGGUACAUUACCUUGUA
847	UCUACCAA AGAA GUUG ACCAGAGAAACACGUGGUACAUUACCUUGUA
913	AGGAUCUG AGAA GCUA ACCAGAGAAACACGUGGUACAUUACCUUGUA
946	AAGUTGTA AGAA GUUA ACCAGAGAAACACGUGGUACAUUACCUUGUA
1234	CCAAGCCA AGAA GTCU ACCAGAGAAACACGUGGUACAUUACCUUGUA
1275	AUTUCAGA AGAA GCTG ACCAGAGAAACACGUGGUACAUUACCUUGUA
1325	UGCCUUCG AGAA GCAG ACCAGAGAAACACGUGGUACAUUACCUUGUA
1350	CCCCGAUG AGAA CCAG ACCAGAGAAACACGUGGUACAUUACCUUGUA
1534	ACAUAAGA AGAA CCCA ACCAGAGAAACACGUGGUACAUUACCUUGUA
1851	GUCCACCC AGAA GUAG ACCAGAGAAACACGUGGUACAUUACCUUGUA
1880	AGAAUGAA AGAA GCTU ACCAGAGAAACACGUGGUACAUUACCUUGUA

Table 8
Rat ICAM Hairpin Ribozyme/Substrate Sequences
Hairpin Ribozyme Sequence

nt.	Position	Substrate
5	AAAGUGCA AGAA GCGG ACCAGAGAACACACGGUUGGUACAUUACCGUA	CUGCU GCC UGCACUUU
59	GGAGGAGA AGAA GCAU ACCAGAGAACACACGGUUGGUACAUUACCGUA	AUGCU GCC UCUGUCC
84	GGGAUCAC AGAA GCGA ACCAGAGAACACACGGUUGGUACAUUACCGUA	UCGCC GUU GUGAUCC
295	GCACAGUG AGAA GCTG ACCAGAGAACACACGGUUGGUACAUUACCGUA	CAGCA GAC CACUGUC
329	AAGCCGAG AGAA GCGU ACCAGAGAACACACGGUUGGUACAUUACCGUA	ACGCA GUC CUCGGCUU
433	UUCCACCA AGAA GGGC ACCAGAGAACACACGGUUGGUACAUUACCGUA	GCGCU GCC UGGUGGA
626	CAUUCUG AGAA GUGA ACCAGAGAACACACGGUUGGUACAUUACCGUA	UCAUC GUU CAGGAUG
806	UCUCCAGG AGAA GCAU ACCAGAGAACACACGGUUGGUACAUUACCGUA	AUGCU GAC CCUGGAGA
849	UCCACUGA AGAA GUGG ACCAGAGAACACACGGUUGGUACAUUACCGUA	CCACU GCC UCAGUGGA
915	AGGGUCUG AGAA GCCA ACCAGAGAACACACGGUUGGUACAUUACCGUA	UGGCG GAC CAGACCCU
1182	ACCUCAA AGAA GGAG ACCAGAGAACACACGGUUGGUACAUUACCGUA	CUGCC GCC UGGAGGU
1307	AUGUAAGA AGAA GCU ACCAGAGAACACACGGUUGGUACAUUACCGUA	CAGCA GAC UCTUACAU
1357	UGCUUUCC AGAA GCG ACCAGAGAACACACGGUUGGUACAUUACCGUA	CUGCA GCC GGAAGCA
1382	UCCCAGAU AGAA GCGG ACCAGAGAACACACGGUUGGUACAUUACCGUA	CCGCU GCC UAUCCGGA
1858	GCCCCACCA AGAA GUAG ACCAGAGAACACACGGUUGGUACAUUACCGUA	CUACA GCC UGGUGGGC
1887	AGAAGGAA AGAA GCU ACCAGAGAACACACGGUUGGUACAUUACCGUA	AGGCU GAC UUCCUUCU
2012	GAGUUGGG AGAA GUGU ACCAGAGAACACACGGUUGGUACAUUACCGUA	ACACU GUC CCCAACUC
2303	AGACUCCA AGAA GUGG ACCAGAGAACACACGGUUGGUACAUUACCGUA	CCACA GCC UGGAGGUU
2539	CCUCCAC AGAA GCU ACCAGAGAACACACGGUUGGUACAUUACCGUA	AAGCU GUU GUGGGAGG

Table 9: Rat ICAM HH Ribozyme Target Sequence

nt. Position	HH Target sequence	nt. Position	HH Target Sequence
11	GAUCCAAU U CACACUGA	394	GUGGUGCU U CUGAACAG
23	GCUGACUU C CUUCUCUA	420	GCACCCCCU C CCAGCGCA
26	GAACUGCU C UUCCUCUU	425	CCUCGGCU U CGGCCACC
31	CCUCUGCU C CUGGUCCU	427	UCCCGGUU U AAAAACCA
34	CUGAAGCU C AGAUAUAC	450	AAGAACCU C AUCCUGCG
40	CUCUAGGU A CAAGCCCC	451	GGGUACUU C CCCCAGGC
48	GAGAACCU C GGCCUGGG	456	CUCGGCJU C UGCCACCA
54	CCCCGOCU C CCUGAGCC	495	GCCACCAU C ACUGUGUA
58	CGUGGCCU U UAGCUOCC	510	GUGCUGCU C CGUGGGAA
64	CAAUGGCCU U CAACCCGU	564	GAAAUGU U CCAACAC
96	CCUCUGCU C CUGGUCCU	592	GGGAGUAU C ACCAGGGA
102	CUCCUGGU C CUGGUCGC	607	GAGCCAAU U UCUAUGC
108	GGACTUGCU U GGGGAACU	608	AGCCAAU U CCUGCU
115	UCCUACCU U UGUUCCCA	609	GCCAAUUU C UCAUGCUU
119	GACACUGU C CCCAACUC	611	CAAUUCU C AUGCUUCA
120	GUUGUGAU C CCCGGGCC	656	GUACUGU U CAGAAUG
146	CCAGACCU U GGAACUCC	657	UCACTUGUU C AAGAAGGU
152	ACCCGGCU C CACCUAA	668	GAACUGCU C UUCCUCUU
158	AUUUCUUU C ACCAGUCA	677	GCACCCCCU C CCAGCGCA
165	UGAACAGU A CUUCCCC	684	AGGCAGCU C CGGACUUU
168	GAAGCCUU C CUGCCUCG	692	CCAGACCU U GGAACUCC
185	GGGUGGGAU C CGUGCAGG	693	CGGACUUU C GAUCUUCC
209	CAGCCCCU A AUCUGACC	696	GCCUGUUU C CUGCCUCU
227	GACCAAGU A ACUGUGAA	709	CAGCAUUU A CCCCUCAC
230	CAAGCUGU U GUGGGAGG	720	CUACAAACU U TUCAGCUC
237	CUGAAGCU C GACACCCC	723	CAACUUUU C AGCUCCCCA
248	GGCCCCCU A CCUUAGGA	735	CUCCUGGU C CUGGUCGC
253	CACUGCCU C AGUGGAGG	738.	UCCUGCCU C GGGGUGGA
263	GAGCCAAU U UCUCAUUC	765	ACUGUGCU U UGAGAACU
267	GAAGCCUU C CUGCCUOG	769	UCUUGUGU U CCCUGGAA
293	GAAGCUCU U CAAGCUGA	770	CUUGUGUU C CCUGGAAG
319	CGGAGGAU C ACAAACGA	785	AGGCCUGU U UCCUGCCU
335	ACUGUGCU U UGAGAACU	786	GGOCUGUU U CCUGCCUC
337	UGUGCUAU A UGGUCCUC	792	CUCCUGGU C CUGGUCGC
338	AAGCUCUU C AAGCUGAG	794	UCCUGECU C UGAAGCUC
359	CAOGCAGU C CUCGGCUU	807	GCUCAGAU A UACCUUGGA
367	CAAUGGCCU U CAACCCGU	833	CCUGGGGU U GGAGACUA
374	UUACCCCU C ACCACCU	846	CUGACAGU U AUUUAUUG
375	AGAAGCCU U CCUGGCCU	851	GCUCACCU U UAGCAGCU
378	ACCCACCU C ACAGGGUA	863	CAAUGGCCU U CAACCCGU
386	CGCUGUGU U UGGAGCU	866	CCAUCCUU C CUCUGACA

867	GACCACCU C CCCACCUA	1421	GGGUACUU C CCCCAGGC
869	CUCUUCCU C UUGCGAAG	1425	ACCCACCU C CUCUGGCC
881	AAUGGCUU C AACCCGUG	1429	AUACUUGU A GCCUCAGG
885	GACCAAGU A ACUGUGAA	1444	AGAAGGCU C AGGAGGAG
933	UGUGUADU C GUUCCAG	1455	GGGAGUAU C ACCAGGGGA
936	GCAGAGAU U UUGUGUCA	1482	AGGGUACU U CCCCCCAGG
978	UUGAGAAU C UACAACUU	1484	ACUGCUCU U CCUCUUGC
980	GAGAAUCU A CAACUUTU	1493	CCUGGGGU U GGAGACTUA
986	CUACAACU U UUCAGCUC	1500	CGUGAAAU U AUGGUCAA
987	UACAAACU U UCAGCUCC	1503	AAAAAUGU U CCIAACCAC
988	ACRACUUU U CAGCUOCC	1506	UGGGUCAU A AUJUGUUGG
1005	UUOGUGAU C GUGGOGUC	1509	GCCACCAU C ACUGUGUA
1006	GUGGGAGU A UCACCAGG	1518	GUCCUGGU C GCGGUJUGU
1023	CCGGAGGU C UCAGAAGG	1530	ACCUGGGU C AUAAUJUGU
1025	GGAGGUUCU C AGAAGGGG	1533	CUGAUCAU U GCGGGCUU
1066	CCUACCUU U GUUCCCAA	1551	GGGGCCCCU C UGCUCUGUA
1092	AGAGGGGU C UCAGCAGA	1559	UGGGAAGU C CCUGUUUA
1093	AGGGGAAU C CAGCCCCU	1563	UCCUACCU U UGUUCCCA
1125	CCCCAACU C UUGUUGAU	1565	UUACACCU A UUACCGCC
1163	ACGACGCU U CUUUVGCU	1567	ACACCUAU U ACGOCAG
1164	CGACGCUU C UUUUGCUC	1584	AGGAAGAU C AGGAUATA
1166	ACGCUUCU U UUGCUCUG	1592	CAGCAUAI A CAAGUJAC
1172	CUUUUGCU C UGCGGOCU	1599	UACAAGUU A CAGAAGGC
1200	AUCCAIIU C ACACUGAA	1651	CCCOGOCU C CCUGAGCC
1201	UUGGGCUU C UCCACAGG	1661	CUGCACUU U GCCCUGGU
1203	GGGCUUCU C CACAGGUC	1663	GAACAGAU C AAUGGACA
1227	UUGGAACU C CAUGUGCU	1678	GAGAACCU C GGCGTUGGG
1228	GOGGGCUU C GUGAUOGU	1680	GGGCUCU C CACAGGUC
1233	CUCCUGGU C CUGGUOGC	1681	GGGCUGUU U CCUGCCUC
1238	UGUGCUAU A UGGUOUCU	1684	CUGCUOGU A GACCUCUC
1264	GGAAAGAU C AIAOAGGGU	1690	CCCCACCU A CAUACAUU
1267	GUACACUGU U CAAGAAGG	1691	COGACUU U CGAUCUUC
1294	CAGAGAUU U UGUGUCAG	1696	CUCCUGGU C CUGGUOGC
1295	AGAGGGGU C UCAGCAGA	1698	UCAGAIIA U CCUGGAGA
1306	AGCAGACU C UUACAUUC	1737	GAUCACAU U CAOGGUGC
1321	AACAGAGU C UGGGGAAA	1750	GUCCAUUU A CACCUAUU
1334	GUAUUOGU U CCCAGAGC	1756	CCUCUGCU C CUGGUCCU
1344	UCCGGUGCU C AGGUADCC	1787	GAGAACCU C GGCGTUGGG
1351	UCAGGOCU A AGAGGACU	1790	GACACUGU C CCCAACUC
1353	UAGCAGCU C AACAAUGG	1793	AUGGUCCU C ACCUGGAC
1366	AGGGUACU U CCCCCAGG	1797	UCCCUGUU U AAAAACCA
1367	GGGUACUU C CCCCGAGC	1802	GCUCAGAU A UACCUUGA
1368	GAUGGUGU C COGCTUGCC	1812	AACAGAGU C UGGGGAAA
1380	CUGOCUAI C GGGGAUGGU	1813	GGGGCUU C GUGAUCGU
1388	UGGAGACU A ACUGGAUG	1825	GCCACCAU C ACUGUGUA
1398	CUGGCUGU C ACAGGACA	1837	ACCCACCU C ACAGGGUA
1402	CUGUGCUU U GAGAACUG	1845	AGAGGACU C GGAGGGGC
1408	UUOGUGAU C GUGGOGUC	1856	CCCCUAAU C UGACCUGC
1410	CGAACAUU C GAGUGGAC	1861	CAUGUGCU A UAUUGGUCC

1865	UAUCCGGU A GACACAAG	2198	GAAUGUCU C CGAGGUCA
1868	UCACCGAGU C AUAAUAAA	2199	AGACUCUU A CAUGGCCAG
1877	ACAGUACU U CCCCCCAGG	2200	GGGUACUU C CCCCAGGC
1901	CJAAAACU C AAAGGUACA	2201	GGGCUUUCU C CACAGGUC
1912	GAACAGAU C AAUGGACA	2205	UUUUGUGUJ C AGCCACTG
1922	AUGUAAGU U AUUGCCUA	2210	UGGAGACU A ACUGGAUG
1923	UGGACGCCU C ACCUUUAG	2220	GAGAACCU C GGCGUUGGG
1928	GCUCAGAU A UACCUGEA	2224	ACAUACAU U CCUACCCU
1930	UGGAGACU A ACTGGGAUG	2226	CUGGACCU C AGGCCACA
1964	AGAGAUU U GGUCAGC	2233	UCALGCUU C ACAGACU
1983	GAGAACCU C GGCUGGG	2242	ACACAGCU C UCAGTACU
1996	UGGAAGCU C UUCAAGCU	2248	CUCCUGGU C CGGGUCGC
2005	ADGUAAGU U AUUGCCUA	2254	AUCCAAU C ACACUGUA
2013	CSCUGCCU A UCGGGGAUG	2259	GAUCACAU U CAUGGGUC
2015	CUGCCJAU C GGGGAUGGU	2260	AUCACAUU C ACGGUGCU
2020	UAUUGAGU A CCCUGUAC	2266	AUCAGGAAU A UACAAGUU
2039	CGGAGGAU C ACAAAACGA	2274	GAGCAGGU U AACAUUGUA
2040	CCUGACCU C CUGGAGGU	2279	GGAAAGAU C AUACGGGU
2057	CUGGUCCU C CAAUGGCC	2282	ACAGUUAU U UAUUGAGU
2061	GCGUCCAU U UACACCUA	2288	GGCCUUGGU C CUCCAUG
2071	AUACUUGU A GCGUCAGG	2291	CAGGAUAU A CAAGUAC
2076	UGUAGGCCU C AGGCCUAA	2321	GGAAAGAU C AUACGGGU
2097	CCAACUCU U GUUGAUGU	2338	UUGGGCUU C UCCCAAGG
2098	CCUGACCU C CUGGAGGU	2339	GGGUACUU C CCCCAGGC
2115	UUCOGACU A GGGUCCUG	2341	GGGCGUGU C GGUGCUCA
2128	AGUGCTGU A CCAUGAUC	2344	CTUGCUCGU A GACCUUC
2130	GCCUGGUU C CUGCCUCU	2358	CCCUGCCU C CUCCCCACA
2145	CCAACUCU U GUUGAUGU	2359	CCAUCCAU C CCACAGAA
2152	UUGAGAAU C UACAACUU	2360	CUUGUGUU C CCUGGAAG
2156	UGACAGUU A UUUAUUGA	2376	GAACUGCU C UUCCUCUU
2158	UGAUGUAU U UAUAAA	2377	GACUUCCU U CUCUADUA
2159	GAUGUAU U AUUAUUC	2378	GCUGAUUU C UUUCACGA
2160	AUGUAUUU A UUAUUUCA	2379	CUGCUCUU C CUCUUGCG
2162	ACAUUCCU A CCUUUUGUU	2380	UGAUUUCU U UCAOGAGU
2163	UAUUAUUAU A AUUCAGAG	2382	AUUUUCUUU C ACGAGUCA
2166	UGAUGUAU U UAUUAUUAU	2384	UAUCCGGU A GACACAAG
2167	GAUGUAU U AUUAUUC	2399	UAAAUCU A UGUGGACG
2170	GUAUUUAU U AAUUCAGA	2401	UGUGCUAU A UGGUCCUC
2171	CAGUUAUU U AUUGAGUA	2411	CAAUUUCU C AUGCUUCA
2173	UGUGCUAU A UGGUCCUC	2417	AUCAGGAAU A UACAAGUU
2174	UCUCUAIUU A CCCCCUGCU	2418	UCAUGCUU C ACAGAACU
2175	AUUAUUCU C ACGAGUCA	2425	UUUAUUAU U CAGAGUUC
2176	GAAAAUGU U CCAACCAC	2426	CCUGGGGU U GGAGACUA
2183	UGACAGUU A UUUAUUGA	2433	UCAGAGUU C UGACAGUU
2185	ACAGUUAU U UAUUGAGU	2434	CGGAGGAU C ACAAACGA
2186	CAGUUAUU U AUUGAGUA	2448	UGAACACGU A CUUCCCCC
2187	AGUUAUUU A UUGAGUAC	2449	GAAGCCUU C CUGCCUCG
2189	UUAUUUUAU U GAGUACCC	2451	GGCCUUGGU U CCUGOCUC
2196	CUGACAGU U AUUAUUG	2452	CCUGUUU C CUGCCUCU

2455	ACAUUCCU A CCUUUGUU	2761	OGGACUUU C GAUCUUCC
2459	CCCUGOCU C CUCCCCACA	2765	CUUUUGCU C UGCAGGCCU
2460	CCUACCUU U GUUCCCAA	2769	UUCUCUAU U ACCCCUGC
2479	UUACACCU A UUACCGCC	2797	CGUGAAAU U AUGGUCAA
2480	GUCCGCCGU U GUGAUCCC	2803	CUCAUGCU U CACAGAAC
2483	ACCUUUGU U CCCAAUGU	2804	UCAUGCUU C ACAGAACU
2484	CCUUUGUU C CCAAUGUC	2813	GTUCCCCAU C CUGACCCU
2492	GACCACCU C CCCACCUA	2815	CGGACUUU C GAUTUJCC
2504	ACCUACAU A CAUCCUA	2821	CCUGACCU C CUGGAGGU
2508	ACAUACAU U CCUACCUU	2822	UACAAACUU U UCAGCUCC
2509	CAUACAUU C CJACCUUU	2823	CAACUUUU C AGCUCCCA
2510	GUCCAUUU A CACCUAU	2829	UCGGUGCU C AGGJAUCC
2520	ACCUUUGU U CCCAAUGU	2837	CACAGGGU A CUUCCCCC
2521	CCUUUGUU C CCAAUGUC	2840	GCACCCCCU C CCAGCGCA
2533	ACAGCAUU U ACCCCCUA	2847	UUACCCCCU C ACCCACCU
2540	UCGGUGCU C AGGUAUCC	2853	UUCGAUCU U CCGACUAG
2545	AGGCAGCU C CGGACUUU	2860	UCUUGUGU U CCCUGGAA
2568	CAGAGAUU U UGUGUCAG	2872	GGGCCUGU C GGUGCUCA
2579	CCUGCACTU U UGCCUGG	2877	UGGAGUCU C CCAGCACC
2585	CUGCUOGL A GACCUCUC	2899	AGGCAGCU C CGGACUUU
2588	UGOCUOCU C CCACAGCC	2900	GGCUGACU U CCUUCUCU
2591	CUCUUCOCU C UUGCGAAG	2904	GAACUGCU C UUCCUCUU
2593	UCUCUAAU A CCCCCUGCU	2905	GGCUGACU U CCUUCUCU
2596	CUCCUGGU C UGGUOGLC	2906	GUUGAUGU A UUUAUUAA
2601	UGUGCUAU A UGGUOUCUC	2907	CUGCUCUU C CUCUUGCG
2602	GUCCUGGU C GCGGUGGU	2908	UGAUGUAU U UAUUAAUU
2607	GGGGGAGU A UCACCAGG	2909	GAACUGCU C UUCCUCUU
2608	CUUUAGCU C CGGUGGGA	2910	ACUUCUU C UCUAUUAC
2609	UGGAGACU A ACUGGAUG	2911	UUCCUUU C UAUUACCC
2620	UCAGAGUU C UGACAGUU	2912	AUGUAAUU A UUAAUUCA
2626	CUCUCAGU A GUGCUGCU	2913	UGUGUAUU C GUUCCCAG
2628	UACAACUU U UCAGCUCC	2914	GUAUUUAU U AAUCAGA
2635	UCACAGAU C CAAUUCAC	2915	UAUUUAU A AUUCAGAG
2640	GCUCAGGU A UCCAUCCA	2916	CUCUUCUU C UUGCGAAG
2641	CCCCACCU A CAUACAUU	2917	CUUCCUCU U GCGAAGAC
2642	GCUGUUU C CUGCCUCU	2918	AUUUCUUU C ACGAGUCA
2653	CCACAGGU C AGGGUGCU	2919	UUUUGUGU C AGCCACUG
2659	AGAAGGGU C CUGCAAGC	2931	GAUGGUGU C CGCTUGCC
2689	ACUAGGGU C CUGAACU	2933	UGGAGUCU C CCAGCACC
2691	UCAGGCCU A AGAGGACU	2941	CAGUACUU C CCCCAGGC
2700	AGGGUACU U CCCCCAGG	2951	ACCAUGCU U CCUCUGAC
2704	GACCACCU C CCCACCUA	2952	CGGGACUU U CGAUCUUC
2711	CCCUACCU U AGGAAGGU	2955	UGCUCUCCU C UGACAUU
2712	CCUACCUU A GGAAGGUG	2956	CUUUCCUU U GAAUCAAU
2721	GGAAAGAU C AUACGGGU	2961	UUUUGUGU C AGCCACUG
2724	AAGAUCAU A CGGGUUG	2962	UGUGUAUU C GUUCCCAG
2744	GGGUGGAGU C CGUGCAGG	2965	CUUUGAAU C AAUAAAGU
2750	GUCCCCUGU U UAAAAACC	2966	UGGAAGCU C UUCAAGCU
2759	GACGAACU A UCGAGUGG	2969	GAAUCAAU A AAGUUUUA

2975 UGGAAGCU C UUCAAGCU
2976 UAUAUUGGU C CTUACCCUG
2977 GAAGCUCU U CAAGCUGA

Table 10: Rat ICAM HH Ribozyme Sequences

nt. Position	Rat HH Ribozyme Sequence
11	UCAGUGUG CUGAUGAGGCCGAAAGGCCGAA AUUGGAUC
23	UAGAGAAC CUGAUGAGGCCGAAAGGCCGAA AAGUCAGC
26	AAGAGGAA CUGAUGAGGCCGAAAGGCCGAA ACCAGUUC
31	AGGACCAAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAGG
34	GUUAUCU CUGAUGAGGCCGAAAGGCCGAA AGCTUCAG
40	GGGGCUUG CUGAUGAGGCCGAAAGGCCGAA ACCUUGAG
48	CCCAGGCC CUGAUGAGGCCGAAAGGCCGAA AGGUUCDC
54	GGCUCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCGGGG
58	GGGAGCTA CUGAUGAGGCCGAAAGGCCGAA AGGCACGG
64	ACGGGUUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUUG
96	AGGACCAAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAGG
102	GCGACCAAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGAG
108	AGUCCCCC CUGAUGAGGCCGAAAGGCCGAA AGCAGUCC
115	UGGGAAACA CUGAUGAGGCCGAAAGGCCGAA AGGUAGGA
119	GAGUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGUGUC
120	GGGGGGGG CUGAUGAGGCCGAAAGGCCGAA AUCAACAC
146	GGAGGUCC CUGAUGAGGCCGAAAGGCCGAA AGGUUCGG
152	UUGAGGUG CUGAUGAGGCCGAAAGGCCGAA AGGCGGGU
158	UGACTUCGU CUGAUGAGGCCGAAAGGCCGAA AAAGAAA
165	GGGGGAAG CUGAUGAGGCCGAAAGGCCGAA ACUGUUCA
168	CGAGCCAG CUGAUGAGGCCGAAAGGCCGAA AAGGUUC
185	CCUCCAC CUGAUGAGGCCGAAAGGCCGAA AUCCACCC
209	GGUCAGAU CUGAUGAGGCCGAAAGGCCGAA AGGGCUG
227	UUCACAGU CUGAUGAGGCCGAAAGGCCGAA ACUUGGUC
230	CCUCCCCAC CUGAUGAGGCCGAAAGGCCGAA ACAGCUUG
237	GGGGUGUC CUGAUGAGGCCGAAAGGCCGAA AGCUUCAG
248	UCCUAAGG CUGAUGAGGCCGAAAGGCCGAA AGGGGGCC
253	CCUCCACU CUGAUGAGGCCGAAAGGCCGAA AGGCAGUG
263	GCAUGAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGCUC
267	CGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUUC
293	UCAGCUUG CUGAUGAGGCCGAAAGGCCGAA AGAGCUUC
319	UCGUUUUGU CUGAUGAGGCCGAAAGGCCGAA AUCCUCG
335	AGUUCUCA CUGAUGAGGCCGAAAGGCCGAA AGCACAGU
337	GAGGACCA CUGAUGAGGCCGAAAGGCCGAA AUAGCACA
338	CUCAGCUU CUGAUGAGGCCGAAAGGCCGAA AAGAGCUU
359	AAGCCGAG CUGAUGAGGCCGAAAGGCCGAA ACUGCGUG
367	ACGGGUUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUUG
374	AGGUGGGU CUGAUGAGGCCGAAAGGCCGAA AGGGGUAA
375	GAGGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCUUCU
378	UACCCUGU CUGAUGAGGCCGAAAGGCCGAA AGGUGGGU
386	AGCUCCAA CUGAUGAGGCCGAAAGGCCGAA ACACAGCG

394 CUGUUCAG CUGAUGAGGCCGAAAGGCCGAA AGCACCCAC
 420 UGCUGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUGC
 425 GGUGGCCAG CUGAUGAGGCCGAAAGGCCGAA AGCCGAGG
 427 UGGUUUUU CUGAUGAGGCCGAAAGGCCGAA AACAGGGA
 450 CGCAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGUUCUU
 451 GCCUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUACCC
 456 UGGUGGCA CUGAUGAGGCCGAAAGGCCGAA AAGCCGAG
 495 UACACAGU CUGAUGAGGCCGAAAGGCCGAA AUGGUGGC
 510 UUCCCACG CUGAUGAGGCCGAAAGGCCGAA AGCAGCAC
 564 GUGGUJUGG CUGAUGAGGCCGAAAGGCCGAA ACAUUUUC
 592 UCCCUGGU CUGAUGAGGCCGAAAGGCCGAA AUACUCCC
 607 GCAUGAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGCUC
 608 AGCAUGAG CUGAUGAGGCCGAAAGGCCGAA AAUUGGU
 609 AAGCAUGA CUGAUGAGGCCGAAAGGCCGAA AAAUUGGC
 611 UGAAGCAU CUGAUGAGGCCGAAAGGCCGAA AGAAAIIUG
 656 CAUUCUUG CUGAUGAGGCCGAAAGGCCGAA ACAGUGAC
 657 ACAUUCUU CUGAUGAGGCCGAAAGGCCGAA AACAGUGA
 668 AAGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGCAGUUC
 677 UGGCGUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUGC
 684 AAAGUOOG CUGAUGAGGCCGAAAGGCCGAA AGCUGCCU
 692 GGAGUUC CUGAUGAGGCCGAAAGGCCGAA AGGUUCUGG
 693 GGAAGAUU CUGAUGAGGCCGAAAGGCCGAA AAAGUCCG
 696 AGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGGC
 709 GUGAGGGG CUGAUGAGGCCGAAAGGCCGAA AAAUGCUG
 720 GAGCUGAA CUGAUGAGGCCGAAAGGCCGAA AGUUGUAG
 723 UGGGAGCU CUGAUGAGGCCGAAAGGCCGAA AAAAGUUG
 735 GCGACCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGAG
 738 UCCACCCC CUGAUGAGGCCGAAAGGCCGAA AGGCAGGA
 765 AGUUCUCA CUGAUGAGGCCGAAAGGCCGAA AGCACAGU
 769 UUCCAGGG CUGAUGAGGCCGAAAGGCCGAA ACACAAGA
 770 CUCUCCAGG CUGAUGAGGCCGAAAGGCCGAA AACACAAG
 785 AGGCAGGA CUGAUGAGGCCGAAAGGCCGAA ACAGGCCU
 786 GAGGCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGGCC
 792 GCGACCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGAG
 794 GAGCUUCA CUGAUGAGGCCGAAAGGCCGAA AGGCAGGA
 807 UCCAGGUA CUGAUGAGGCCGAAAGGCCGAA AUCUGAGC
 833 UAGUCUCC CUGAUGAGGCCGAAAGGCCGAA ACCCCAGG
 846 CAAUAAA CUGAUGAGGCCGAAAGGCCGAA ACUGUCAG
 851 AGCUGCUA CUGAUGAGGCCGAAAGGCCGAA AGGUGAGC
 863 ACGGGUUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUUG
 866 UGUCAGAG CUGAUGAGGCCGAAAGGCCGAA AAGCAUGG
 867 UAGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUUGGC
 869 CUUCGCAG CUGAUGAGGCCGAAAGGCCGAA AGGAAGAG
 881 CACGGGUU CUGAUGAGGCCGAAAGGCCGAA AAGCCAUU
 885 UUCACAGU CUGAUGAGGCCGAAAGGCCGAA ACUUGGUC
 933 CUGGGAAC CUGAUGAGGCCGAAAGGCCGAA AAUACACA
 936 UGACACAA CUGAUGAGGCCGAAAGGCCGAA AUCUCUGC
 978 AAGUUGUA CUGAUGAGGCCGAAAGGCCGAA AUUCUCAA
 980 AAAAGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUUCUC

986 GAGCTUGAA CUGAUGAGGCCGAAAGGCCGAA AGUUGUAG
 987 GGAGCUGA CUGAUGAGGCCGAAAGGCCGAA AAGUUGUA
 988 GGGAGCUG CUGAUGAGGCCGAAAGGCCGAA AAAGUUGU
 1005 GACGCCAC CUGAUGAGGCCGAAAGGCCGAA AUCAACGAA
 1006 CCUGGUGA CUGAUGAGGCCGAAAGGCCGAA ACUCCAC
 1023 CCUUUCUGA CUGAUGAGGCCGAAAGGCCGAA ACCUCCGG
 1025 CCCCCUUCU CUGAUGAGGCCGAAAGGCCGAA AGACCUCC
 1066 UUGGAAC CUGAUGAGGCCGAAAGGCCGAA AAGGUAGG
 1092 UCUGCUGA CUGAUGAGGCCGAAAGGCCGAA ACCCCCTCU
 1093 AGGGCUG CUGAUGAGGCCGAAAGGCCGAA AUUCCCCU
 1125 AUCAACAA CUGAUGAGGCCGAAAGGCCGAA AGUUGGGG
 1163 AGCAAAAG CUGAUGAGGCCGAAAGGCCGAA AGCGUOGU
 1164 GAGCAAAA CUGAUGAGGCCGAAAGGCCGAA AACGCGUG
 1166 CAGAGCRA CUGAUGAGGCCGAAAGGCCGAA AGAACGCU
 1172 AGGCCGCA CUGAUGAGGCCGAAAGGCCGAA AGCAAAAG
 1200 UUCAGUGU CUGAUGAGGCCGAAAGGCCGAA AAUUGGAU
 1201 CCUGUGGA CUGAUGAGGCCGAAAGGCCGAA AAGCCCAA
 1203 GACCUGUG CUGAUGAGGCCGAAAGGCCGAA AGAACGCC
 1227 AGCACAUG CUGAUGAGGCCGAAAGGCCGAA AGUUCCAA
 1228 ACGAUACAC CUGAUGAGGCCGAAAGGCCGAA AAGCCCGC
 1233 GOGACCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGAG
 1238 GAGGACCA CUGAUGAGGCCGAAAGGCCGAA AUAGCACA
 1264 ACCCGUAU CUGAUGAGGCCGAAAGGCCGAA AUUUUUC
 1267 CAUUCUUG CUGAUGAGGCCGAAAGGCCGAA ACAGUGAC
 1294 CUGACACA CUGAUGAGGCCGAAAGGCCGAA AUUCUCUG
 1295 UCUGCUGA CUGAUGAGGCCGAAAGGCCGAA ACCCCUCU
 1306 GCAUGUAA CUGAUGAGGCCGAAAGGCCGAA AGUCUGCU
 1321 UUUCCCCA CUGAUGAGGCCGAAAGGCCGAA ACUCUGUU
 1334 GCUCUGGG CUGAUGAGGCCGAAAGGCCGAA AGGAUAC
 1344 GGAAUACCU CUGAUGAGGCCGAAAGGCCGAA AGCACCGA
 1351 AGUCCUCU CUGAUGAGGCCGAAAGGCCGAA AGCCCTUGA
 1353 CCAUUGUU CUGAUGAGGCCGAAAGGCCGAA AGCUGCUA
 1366 CCUGGGGG CUGAUGAGGCCGAAAGGCCGAA AGUACCCU
 1367 GCGUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUACCC
 1368 GGCAGCGG CUGAUGAGGCCGAAAGGCCGAA ACACCAUC
 1380 ACCAUCCC CUGAUGAGGCCGAAAGGCCGAA AUAGGCAG
 1388 CAUCCAGU CUGAUGAGGCCGAAAGGCCGAA AGUCUCCA
 1398 UGUCCUGU CUGAUGAGGCCGAAAGGCCGAA ACAGCCAG
 1402 CAGUUCUC CUGAUGAGGCCGAAAGGCCGAA AAGCACAG
 1408 GACGCCAC CUGAUGAGGCCGAAAGGCCGAA AUCAAGAA
 1410 GUCCACUC CUGAUGAGGCCGAAAGGCCGAA AUAGUUCG
 1421 GCCUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUACCC
 1425 AGCCAGAG CUGAUGAGGCCGAAAGGCCGAA AGGUGGGU
 1429 CCUGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAGUAU
 1444 CUCCUCCU CUGAUGAGGCCGAAAGGCCGAA AGCCUUCU
 1455 UCCCCUGGU CUGAUGAGGCCGAAAGGCCGAA AUACUCCC
 1482 CCUGGGGG CUGAUGAGGCCGAAAGGCCGAA AGUACCCU
 1484 GCAAGAGG CUGAUGAGGCCGAAAGGCCGAA AGAGCAGU
 1493 UAGUCUCC CUGAUGAGGCCGAAAGGCCGAA ACCCCAGG

1500 UUGACCAU CUGAUGAGGCCGAAAGGCCGAA AUUCACG
 1503 GUGGUUGG CUGAUGAGGCCGAAAGGCCGAA ACUUUUUC
 1506 CCAACAAU CUGAUGAGGCCGAAAGGCCGAA AUGACCCA
 1509 UACACAGU CUGAUGAGGCCGAAAGGCCGAA AUGGUGGC
 1518 ACAACGGC CUGAUGAGGCCGAAAGGCCGAA ACCAGGAC
 1530 ACAAUUAU CUGAUGAGGCCGAAAGGCCGAA ACCCAGGU
 1533 AAGCCCGC CUGAUGAGGCCGAAAGGCCGAA AUGAUCAG
 1551 UACGAGCA CUGAUGAGGCCGAAAGGCCGAA AGGCCAC
 1559 UAAACAGG CUGAUGAGGCCGAAAGGCCGAA ACUUCCCA
 1563 UGGGAACA CUGAUGAGGCCGAAAGGCCGAA AGGUAGGA
 1565 GGCGGUAA CUGAUGAGGCCGAAAGGCCGAA AGGUGUAA
 1567 CUGGGGU CUGAUGAGGCCGAAAGGCCGAA AUAGGUGU
 1584 UADAUCCU CUGAUGAGGCCGAAAGGCCGAA AUCUUCU
 1592 GUAACTUG CUGAUGAGGCCGAAAGGCCGAA AUAUCCUG
 1599 GCCUUCUG CUGAUGAGGCCGAAAGGCCGAA AACUUGUA
 1651 GGCUCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCCGGG
 1661 ACCAGGGC CUGAUGAGGCCGAAAGGCCGAA AAGUGCAG
 1663 UGUCCAUU CUGAUGAGGCCGAAAGGCCGAA AUCUGUUC
 1678 CCCAGGCC CUGAUGAGGCCGAAAGGCCGAA AGGUUCUC
 1680 GACCUGUG CUGAUGAGGCCGAAAGGCCGAA AGAACCCC
 1681 GAGGCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGGCC
 1684 GAGAGGUC CUGAUGAGGCCGAAAGGCCGAA ACGAGCAG
 1690 AAUGUAUG CUGAUGAGGCCGAAAGGCCGAA AGGUGGGG
 1691 GAAGAUUG CUGAUGAGGCCGAAAGGCCGAA AAGUCCGG
 1696 GCGACCAAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGAG
 1698 UCUCCAAGG CUGAUGAGGCCGAAAGGCCGAA AUAUUGA
 1737 GCACCGUG CUGAUGAGGCCGAAAGGCCGAA AUGUGAUC
 1750 AAUAGGUG CUGAUGAGGCCGAAAGGCCGAA AAAUGGAC
 1756 AGGACCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAGG
 1787 CCAGGGCC CUGAUGAGGCCGAAAGGCCGAA AGGUUCUC
 1790 GAGUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGUGUC
 1793 GUCCAGGU CUGAUGAGGCCGAAAGGCCGAA AGGACCAU
 1797 UGGUUUUU CUGAUGAGGCCGAAAGGCCGAA AACAGGGG
 1802 UCCAGGU CUGAUGAGGCCGAAAGGCCGAA AUCUGAGC
 1812 UUUCCCCA CUGAUGAGGCCGAAAGGCCGAA ACUCUGUU
 1813 ACGAUACAC CUGAUGAGGCCGAAAGGCCGAA AAGCCCGC
 1825 UACACAGU CUGAUGAGGCCGAAAGGCCGAA AUGGUGGC
 1837 UACCCUGU CUGAUGAGGCCGAAAGGCCGAA AGGUGGGU
 1845 GCCCCUCC CUGAUGAGGCCGAAAGGCCGAA AGUCCUCU
 1856 CGAGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUAGGGG
 1861 GGACCAUA CUGAUGAGGCCGAAAGGCCGAA AGCACAU
 1865 CUUGUGUC CUGAUGAGGCCGAAAGGCCGAA ACCGGAA
 1868 AUUUAUUAU CUGAUGAGGCCGAAAGGCCGAA ACUCGUGA
 1877 CCUGGGGG CUGAUGAGGCCGAAAGGCCGAA AGUACUGU
 1901 UGUACCUU CUGAUGAGGCCGAAAGGCCGAA AGUUUUAG
 1912 UGUCCAUU CUGAUGAGGCCGAAAGGCCGAA AUCUGUUC
 1922 UAGGCRAU CUGAUGAGGCCGAAAGGCCGAA ACUUACAU
 1923 CUAAAGGU CUGAUGAGGCCGAAAGGCCGAA AGCGUCCA
 1928 UCCAGGU CUGAUGAGGCCGAAAGGCCGAA AUCUGAGC

1930 CAUCCAGU CUGAUGAGGCGAAAGGCCGAA AGUCUCCA
 1964 CCUGACAC CUGAUGAGGCGAAAGGCCGAA AAACUCU
 1983 CCCAGGCC CUGAUGAGGCGAAAGGCCGAA AGGUUCUC
 1996 AGCUUGAA CUGAUGAGGCGAAAGGCCGAA AGCUUCCA
 2005 UAGGCAAU CUGAUGAGGCGAAAGGCCGAA ACUUAU
 2013 CAUCCCGA CUGAUGAGGCGAAAGGCCGAA AGGCAGCG
 2015 ACCAUCCC CUGAUGAGGCGAAAGGCCGAA AUAGGCAG
 2020 GUACAGGG CUGAUGAGGCGAAAGGCCGAA ACUUAU
 2039 UCGUUUUGU CUGAUGAGGCGAAAGGCCGAA AUCCUUCG
 2040 ACCUCCAG CUGAUGAGGCGAAAGGCCGAA AGGUUCAGG
 2057 AGCCAUUG CUGAUGAGGCGAAAGGCCGAA AGGACCAG
 2061 UAGGUGUA CUGAUGAGGCGAAAGGCCGAA AUGGAOGC
 2071 CCUGAGGC CUGAUGAGGCGAAAGGCCGAA ACAAGUAU
 2076 UUAGGCCU CUGAUGAGGCGAAAGGCCGAA AGCCUACA
 2097 ACAUCAAC CUGAUGAGGCGAAAGGCCGAA AGAGUUGG
 2098 ACCUCCAG CUGAUGAGGCGAAAGGCCGAA AGGUUCAGG
 2115 CAGGACCC CUGAUGAGGCGAAAGGCCGAA AGUCCGAA
 2128 GAUCAUUG CUGAUGAGGCGAAAGGCCGAA ACAGCAU
 2130 AGAGGCAG CUGAUGAGGCGAAAGGCCGAA AAACAGGC
 2145 ACAUCAAC CUGAUGAGGCGAAAGGCCGAA AGAGUUGG
 2152 AAGUUGUA CUGAUGAGGCGAAAGGCCGAA AUUCUAA
 2156 UCAUAAA CUGAUGAGGCGAAAGGCCGAA AACUGUCA
 2158 AAUUAUUA CUGAUGAGGCGAAAGGCCGAA AUACAUCA
 2159 GAAUAAA CUGAUGAGGCGAAAGGCCGAA AAUACACU
 2160 UGAUAAA CUGAUGAGGCGAAAGGCCGAA AAUACAU
 2162 AACAAAGG CUGAUGAGGCGAAAGGCCGAA AGGAUGU
 2163 CUCUGAAU CUGAUGAGGCGAAAGGCCGAA AAUAAAUA
 2166 AAUAAAUA CUGAUGAGGCGAAAGGCCGAA AUACAUCA
 2167 GAAUAAA CUGAUGAGGCGAAAGGCCGAA AAUACAU
 2170 UCUGAAU CUGAUGAGGCGAAAGGCCGAA AAUAAUAC
 2171 UACUCAAU CUGAUGAGGCGAAAGGCCGAA AAUACUG
 2173 GAGGACCA CUGAUGAGGCGAAAGGCCGAA AUAGCCACA
 2174 ACCACGGG CUGAUGAGGCGAAAGGCCGAA AAUAGAGA
 2175 UGACUCGU CUGAUGAGGCGAAAGGCCGAA AAAGAAA
 2176 GUGGUJUG CUGAUGAGGCGAAAGGCCGAA ACAUUUC
 2183 UCAUAAA CUGAUGAGGCGAAAGGCCGAA AACUGUCA
 2185 ACUCAADA CUGAUGAGGCGAAAGGCCGAA AAUACUGU
 2186 UACUCAAU CUGAUGAGGCGAAAGGCCGAA AAUACUG
 2187 GUACUCAA CUGAUGAGGCGAAAGGCCGAA AAUUAACU
 2189 GGGUACUC CUGAUGAGGCGAAAGGCCGAA AAUAAAUA
 2196 CAAUAAA CUGAUGAGGCGAAAGGCCGAA ACUGUCAG
 2198 UGACCUUC CUGAUGAGGCGAAAGGCCGAA AGACAUUC
 2199 CUGGCAUG CUGAUGAGGCGAAAGGCCGAA AAGAGUC
 2200 GCCUGGGG CUGAUGAGGCGAAAGGCCGAA AAGUACCC
 2201 GACCUGUG CUGAUGAGGCGAAAGGCCGAA AGAACCC
 2205 CAGUGGCC CUGAUGAGGCGAAAGGCCGAA ACACAAA
 2210 CAUCCAGU CUGAUGAGGCGAAAGGCCGAA AGUCUCCA
 2220 CCCAGGCC CUGAUGAGGCGAAAGGCCGAA AGGUUCUC
 2224 AAGGUAGG CUGAUGAGGCGAAAGGCCGAA AUGUAOGU

2226 UGUGGCCU CUGAUGAGGCCGAAAGGCCGAA AGGUCCAG
 2233 AGUUCUGU CUGAUGAGGCCGAAAGGCCGAA AACCAUGA
 2242 ACUACUGA CUGAUGAGGCCGAAAGGCCGAA AGCUGUGU
 2248 GCGACCAAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGAG
 2254 UUCAGUGU CUGAUGAGGCCGAAAGGCCGAA AAUUGGAU
 2259 GCACCGUG CUGAUGAGGCCGAAAGGCCGAA AUGUGAU
 2260 AGCACCGU CUGAUGAGGCCGAAAGGCCGAA AAUUGGAU
 2266 AACUUGUA CUGAUGAGGCCGAAAGGCCGAA ACCUGAU
 2274 UACAUGUU CUGAUGAGGCCGAAAGGCCGAA ACCUGUC
 2279 ACCCGUAU CUGAUGAGGCCGAAAGGCCGAA ACCUUUCC
 2282 ACUCAATA CUGAUGAGGCCGAAAGGCCGAA AUAACUGU
 2288 CAUUGGAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGGC
 2291 GUAACTUUG CUGAUGAGGCCGAAAGGCCGAA AUAAUCCUG
 2321 ACCCGUAU CUGAUGAGGCCGAAAGGCCGAA ACCUUUCC
 2338 CCUGUGGA CUGAUGAGGCCGAAAGGCCGAA AAGCCCCA
 2339 GCCUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGUACCC
 2341 UGAGCACC CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
 2344 GAGAGGUC CUGAUGAGGCCGAAAGGCCGAA ACGAGCAG
 2358 UGUGGGAG CUGAUGAGGCCGAAAGGCCGAA AGGCAGGG
 2359 UUCUGUGG CUGAUGAGGCCGAAAGGCCGAA AUUGGAUGG
 2360 CUUCCAGG CUGAUGAGGCCGAAAGGCCGAA AACACAAG
 2376 AAGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGCAGUUC
 2377 UAAUAGAG CUGAUGAGGCCGAAAGGCCGAA AGGAAGUC
 2378 UCGUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAUCAGC
 2379 CGCAAGAG CUGAUGAGGCCGAAAGGCCGAA AAGAGCAG
 2380 ACUCGUGA CUGAUGAGGCCGAAAGGCCGAA AGAAAUC
 2382 UGACUCGU CUGAUGAGGCCGAAAGGCCGAA AAAGAAAU
 2384 CUUGUGUC CUGAUGAGGCCGAAAGGCCGAA ACCGGAUA
 2399 CGUCCACA CUGAUGAGGCCGAAAGGCCGAA AGUAUUUA
 2401 GAGGACCA CUGAUGAGGCCGAAAGGCCGAA AUAGCACA
 2411 UGAAGCAU CUGAUGAGGCCGAAAGGCCGAA AGAAAUG
 2417 AACUUGUA CUGAUGAGGCCGAAAGGCCGAA AUCCUGAU
 2418 AGUUCUGU CUGAUGAGGCCGAAAGGCCGAA AAGCAUGA
 2425 GAACUCUG CUGAUGAGGCCGAAAGGCCGAA AUUAAUAA
 2426 UAGUCUCC CUGAUGAGGCCGAAAGGCCGAA ACCCCAGG
 2433 AACUGUCA CUGAUGAGGCCGAAAGGCCGAA AACUCUGA
 2434 UCGUUUGU CUGAUGAGGCCGAAAGGCCGAA AUCCUCGG
 2448 GGGGAAG CUGAUGAGGCCGAAAGGCCGAA ACUGUUC
 2449 CGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUUC
 2451 GAGGCAGG CUGAUGAGGCCGAAAGGCCGAA AACAGGCC
 2452 AGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGGC
 2455 AACAAAGG CUGAUGAGGCCGAAAGGCCGAA AGGAUGU
 2459 UGUGGGAG CUGAUGAGGCCGAAAGGCCGAA AGGCAGGG
 2460 UUGGGAAC CUGAUGAGGCCGAAAGGCCGAA AAGGUAGG
 2479 GGCGGUAA CUGAUGAGGCCGAAAGGCCGAA AGGUUAA
 2480 GGGAUACAC CUGAUGAGGCCGAAAGGCCGAA ACGGGGAC
 2483 ACAUUGGG CUGAUGAGGCCGAAAGGCCGAA ACGAAAGU
 2484 GACAUUGG CUGAUGAGGCCGAAAGGCCGAA AACPAAGG
 2492 UAGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUGGUC

2504 UAGGAAUG CUGAUGAGGCCGAAAGGCCGAA AUGUAGGU
 2508 AAGGUAGG CUGAUGAGGCCGAAAGGCCGAA AUGUAGGU
 2509 AAAGGUAG CUGAUGAGGCCGAAAGGCCGAA AAUGUAG
 2510 AADAGGUG CUGAUGAGGCCGAAAGGCCGAA AAAUGGAC
 2520 ACAUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAAAGGU
 2521 GACAUUUGG CUGAUGAGGCCGAAAGGCCGAA AACAAAGG
 2533 UGAGGGGU CUGAUGAGGCCGAAAGGCCGAA AAUGCUGU
 2540 GGAAUACC CUGAUGAGGCCGAAAGGCCGAA AGCACCGA
 2545 AAAGUCCG CUGAUGAGGCCGAAAGGCCGAA AGCUCGCC
 2568 CUGACACA CUGAUGAGGCCGAAAGGCCGAA AAUCUCUG
 2579 CCAGGGCA CUGAUGAGGCCGAAAGGCCGAA AGUGGAGG
 2585 GAGAGGUC CUGAUGAGGCCGAAAGGCCGAA ACGAGCAG
 2588 GGCUGUGG CUGAUGAGGCCGAAAGGCCGAA AGGAGGCA
 2591 CUUCGCAA CUGAUGAGGCCGAAAGGCCGAA AGGAAGAG
 2593 AGCAGGGG CUGAUGAGGCCGAAAGGCCGAA AAUAGAGA
 2596 GOGACCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGAG
 2601 GAGGACCA CUGAUGAGGCCGAAAGGCCGAA AUAGCACA
 2602 ACAACGGC CUGAUGAGGCCGAAAGGCCGAA ACCAGGAC
 2607 CCUGGUGA CUGAUGAGGCCGAAAGGCCGAA ACUCCCAC
 2608 UOCCCACGG CUGAUGAGGCCGAAAGGCCGAA AGCUAAAG
 2609 CAUCCAGU CUGAUGAGGCCGAAAGGCCGAA AGUCUCCA
 2620 AACUGUCA CUGAUGAGGCCGAAAGGCCGAA AACUCUGA
 2626 AGCAGCAC CUGAUGAGGCCGAAAGGCCGAA ACUGAGAG
 2628 GGAGCTUG CUGAUGAGGCCGAAAGGCCGAA AAGUJUGUA
 2635 GUGAAUUG CUGAUGAGGCCGAAAGGCCGAA AUCUGUGA
 2640 UGGAUUGA CUGAUGAGGCCGAAAGGCCGAA ACCUGAGC
 2641 AAUGUAG CUGAUGAGGCCGAAAGGCCGAA AGGUGGGG
 2642 AGAGGCAG CUGAUGAGGCCGAAAGGCCGAA AAACAGGC
 2653 AGCACCCU CUGAUGAGGCCGAAAGGCCGAA ACCUGUGG
 2659 GCUUGCAG CUGAUGAGGCCGAAAGGCCGAA ACCCUUCU
 2689 AGCUUCAG CUGAUGAGGCCGAAAGGCCGAA ACCCUAGU
 2691 AGUCCUCU CUGAUGAGGCCGAAAGGCCGAA AGGCCUGA
 2700 CCUGGGGG CUGAUGAGGCCGAAAGGCCGAA AGUACCCU
 2704 UAGGUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUGGUC
 2711 ACCUUCCU CUGAUGAGGCCGAAAGGCCGAA AGGUAGGG
 2712 CACCUUCC CUGAUGAGGCCGAAAGGCCGAA AAGGUAGG
 2721 ACCCGUAU CUGAUGAGGCCGAAAGGCCGAA AUCUUUCC
 2724 CAAACCCG CUGAUGAGGCCGAAAGGCCGAA AUGAUCUU
 2744 CCUGCPACG CUGAUGAGGCCGAAAGGCCGAA AUCCAACCC
 2750 GGUUUUUUA CUGAUGAGGCCGAAAGGCCGAA ACAGGGAC
 2759 CCACUCGA CUGAUGAGGCCGAAAGGCCGAA AGUUCGUC
 2761 GGAAGAUC CUGAUGAGGCCGAAAGGCCGAA AAAGUCCG
 2765 AGGCCGCA CUGAUGAGGCCGAAAGGCCGAA AGCAAAAG
 2769 GCAGGGGU CUGAUGAGGCCGAAAGGCCGAA AUAGAGAA
 2797 UUGACCAU CUGAUGAGGCCGAAAGGCCGAA AUUUCACG
 2803 GUUCUGUG CUGAUGAGGCCGAAAGGCCGAA AGCAUGAG
 2804 AGUUCUGU CUGAUGAGGCCGAAAGGCCGAA AAGCAUGA
 2813 AGGGUCAG CUGAUGAGGCCGAAAGGCCGAA AUGGGAGC
 2815 GGAAGAUC CUGAUGAGGCCGAAAGGCCGAA AAAGUCCG

2821 ACCUCCAG CUGAUGAGGCCGAAAGGCGAA AGGUCAAG
 2822 GGGCUGA CUGAUGAGGCCGAAAGGCGAA AAGUUGUA
 2823 UGGGAGCU CUGAUGAGGCCGAAAGGCGAA AAAAGJUG
 2829 GGAUACCU CUGAUGAGGCCGAAAGGCGAA AGCACCGA
 2837 GGGGGAAG CUGAUGAGGCCGAAAGGCGAA ACCCUGUG
 2840 UGGCCTGG CUGAUGAGGCCGAAAGGCGAA AGGGGUGC
 2847 AGGUGGGU CUGAUGAGGCCGAAAGGCGAA AGGGGUAA
 2853 CUAGUCGG CUGAUGAGGCCGAAAGGCGAA AGAUUCGA
 2860 UUCCAGGG CUGAUGAGGCCGAAAGGCGAA ACRAAAGA
 2872 UGAGCACC CUGAUGAGGCCGAAAGGCGAA ACRCGGCC
 2877 GGUGCTGG CUGAUGAGGCCGAAAGGCGAA AGRCUCCA
 2899 AAAGUCCG CUGAUGAGGCCGAAAGGCGAA AGCTUGCU
 2900 AGAGAAGG CUGAUGAGGCCGAAAGGCGAA AGUCAGCC
 2904 AAGAGGAA CUGAUGAGGCCGAAAGGCGAA AGCAGUTC
 2905 AGAGAAGG CUGAUGAGGCCGAAAGGCGAA AGUCAGCC
 2906 UUAATAAA CUGAUGAGGCCGAAAGGCGAA ACAUCAAC
 2907 CGCAAGAG CUGAUGAGGCCGAAAGGCGAA AAGAGCAG
 2908 AAUUAUA CUGAUGAGGCCGAAAGGCGAA AUACAUCA
 2909 AAGAGGAA CUGAUGAGGCCGAAAGGCGAA AGCAGUUC
 2910 GUAAUAGA CUGAUGAGGCCGAAAGGCGAA AAGGAGU
 2911 GGGUAUUA CUGAUGAGGCCGAAAGGCGAA AGRAGGAA
 2912 UGAAUUA CUGAUGAGGCCGAAAGGCGAA AAUACAU
 2913 CUGGGAAC CUGAUGAGGCCGAAAGGCGAA AAUACACA
 2914 UCUGAAU CUGAUGAGGCCGAAAGGCGAA AUAAAUC
 2915 CUCUGAAU CUGAUGAGGCCGAAAGGCGAA AAUAAAUA
 2916 CUUCGCAA CUGAUGAGGCCGAAAGGCGAA AGGAAGAG
 2917 GUCUUOCG CUGAUGAGGCCGAAAGGCGAA AGAGGAAG
 2918 UGACUCGU CUGAUGAGGCCGAAAGGCGAA AAAGAAA
 2919 CAGUGGCU CUGAUGAGGCCGAAAGGCGAA ACACAAAA
 2931 GGCAGOGG CUGAUGAGGCCGAAAGGCGAA ACACCAUC
 2933 GGUGCTGG CUGAUGAGGCCGAAAGGCGAA AGACUCCA
 2941 GCCUGGGG CUGAUGAGGCCGAAAGGCGAA AAGUACUG
 2951 GUCAGAGG CUGAUGAGGCCGAAAGGCGAA AGCAUGGU
 2952 GAAGAUJC CUGAUGAGGCCGAAAGGCGAA AAGUOOGG
 2955 CCAUGUCA CUGAUGAGGCCGAAAGGCGAA AGGAAGCA
 2956 AUUGAUUC CUGAUGAGGCCGAAAGGCGAA AAGGAAAG
 2961 CAGUGGCU CUGAUGAGGCCGAAAGGCGAA ACACAAAA
 2962 CUGGGAAC CUGAUGAGGCCGAAAGGCGAA AAUACACA
 2965 ACUUUUAU CUGAUGAGGCCGAAAGGCGAA AUUCAAAG
 2966 AGCUUGAA CUGAUGAGGCCGAAAGGCGAA AGCUUCCA
 2969 UAAAACUU CUGAUGAGGCCGAAAGGCGAA AUUGAUUC
 2975 AGCUUGAA CUGAUGAGGCCGAAAGGCGAA AGCUUCCA
 2976 CAGGUGAG CUGAUGAGGCCGAAAGGCGAA ACCAUUA
 2977 UCAGCUUG CUGAUGAGGCCGAAAGGCGAA AGACCUUC

Table 11: Human IL-5 HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	AUGCACU U UCUUUGC	245	AAGAAAU C UUUCAAGG
9	UGCACUU U CUUUGCC	247	GAAACU U UCAGGCC
10	GCACUUU C UUUGCCA	248	AAAUCUU U CAGGGAA
12	ACUUUCU U UGCCAAA	249	AAUCUUU C AGGGAAU
13	CUUUCUU U GCCAAAG	257	AGGGAAU A GGCACAC
36	AGAACGU U UCAGAGC	273	GGAGAGU C AAACUGU
37	GAACGUU U CAGAGCC	291	AGGGGGU A CUGUGGA
38	AACGUUU C AGAGCCA	305	AAAGACU A UUCAAAA
56	GGAUUCU U CGCAUU	307	AGACUAU U CAAAAAC
57	GAUGCUU C UGCAUUU	308	GACUAUU C AAAAACU
63	UCUGCAU U UGAGUUU	316	AAAAACU U GUCCUUA
64	CUGCAUU U GAGUUUG	319	ACUUGU C CUUAAUA
69	UUUGAGU U UGCUAGC	322	UUGUCCU U AAUAAAG
70	UUGAGUU U GCUAGCU	323	UGUCCUU A AUAAAAGA
74	GUUUGCU A GCUCUUG	326	CCUUAUU A AAGAAAU
78	GCUAGCU C UGGGAGC	334	AAGAAAU A CAUUGAC
80	UAGCUCU U GGAGCUG	338	AAUACAU U GACGGCC
91	GCUGGCC A CGUGUAU	380	GGAGAGU A AACCAAU
97	UACGUGU A UGCCAUC	388	AACCAAU U CCTUAGAC
104	AUGCCAU C CCCACAG	389	ACCAAUU C CTAGACU
116	CAGAAAU U CCCACAA	392	AAUUCU A GACUACC
117	AGAAAUU C CCACAAG	397	CUAGACU A CCUGCAA
130	AGUGCAU U GGUGAAA	409	CAAGAGU U UCUUUGGU
145	GAGACCU U GGCACUG	410	AAGAGUU U CUUUGUG
155	CACUGCU U UCUACUC	411	AGAGUUU C UUGGUGU
156	ACUGCUU U CUACUCA	413	AGUUUCU U GGUGUAA
157	CUGCUUU C UACUCAU	419	UUGGUGU A AUGAACAA
159	GCUUUCU A CUCAUCG	437	AGUGGAU A AUAGAAA
162	UUCUACU C AUOGAAC	440	GGAAUAAU A GAAAGUU
165	UACUCAU C GAACUCU	447	AGAAAAGU U GAGACUA
171	UOGAACU C UGCUGAU	454	UGAGACU A AACUGGU
179	UGCUGAU A GCCAAUG	462	AACUGGU U UGUUGCA
192	UGAGACU C UGAGGAU	463	ACUGGUU U GUUGCAG
200	UGAGGGAU U CCUGUUC	466	GGUUUGU U GCAGCCA
201	GAGGAUU C CUGUCC	479	CAAAGAU U UUGGAGG
206	UUCUGGU U CCUGUAC	480	AAAGAUU U UGGAGGA
207	UCCUGUU C CUGUACA	481	AAGAUUU U GGAGGGAG
212	UUCUGU A CAUAAA	497	AGGACAU U UUACUGC
216	UGUACAU A AAAAUCA	498	GGACAUU U UACUGCA
222	UAAAAAU C ACCAACU	499	GACAUUU U ACUGCAG

500	ACAUUUU A CUGCAGU	684	UACUUUU U UCUUAAU
531	AAAGAGU C AGGCCUU	685	ACUUUUU U CUUAAJUU
538	CAGGCCU U AAUUUUC	686	CUUUUUU C UUAAJUU
539	AGGCCUU A AAUUUCA	688	UUUUUCU U AAUUAAC
542	CCUUAAU U UUCAAAU	689	UUUUUCU A UUUACU
543	CUUAAU U UCAAATA	691	UUCUUAAU U UAACUUA
544	UUAAUUU U CAUATA	692	UCUUAAU U AACUJAA
545	UAAUUUU C AAUAAU	693	CUUAAUJU A ACUUAAC
549	UUUCAAU A UAAUUA	697	UUUAACU U AACAUUC
551	UCAAUAU A AAUUAAC	698	UUAACUU A ACAUUC
554	AUAAUAA U UAACUUC	703	UUAACAU U CUGJAAA
555	UAAUAAU U AACUUCA	704	UAACAUU C UGUAAAA
556	AUAAUUU A ACUUCAG	708	AUUCUGU A AAAUGUC
560	UUUAACU U CAGAGGG	715	AAAAGU C UGUUAAC
561	UUAACUU C AGAGGGA	719	UGUCUGU U AACUUA
573	GGAAAGU A AAUAAU	720	GUCUGUU A ACUUAU
577	AGUAAAU A UUUCAGG	724	GUUAACU U AAUAGUA
579	UAAAUAU U UCAGGCA	725	UUAACUU A AAUAGUA
580	AAAUAAU U CAGGCAU	728	ACUUAU A GUAAUUA
581	AAUAAUU C AGGCAUA	731	UAUAGU A UUUAGUA
588	CAGGCAU A CUGACAC	733	UAUAGUA U UAUAGAA
597	UGACACU U UGCCAGA	734	UAGUAU U AUGAAAU
598	GACACUU U GCCAGAA	735	AGUADUU A UGAAUAG
611	AAAGCAU A AAAUUCU	745	AAADGGU U AAGAAUU
616	AUAAAUAU U CUUAAA	746	AAUUGGU A AGAAUJJ
617	UAAAUAU C UUAAAUA	752	UAAGAAU U UGGUAAA
619	AAAIJUCU U AAAUAAU	753	AAGAAUJU U GGUAUAA
620	AAUUCUU A AAAUATA	757	AUJUGGU A AAUUAGU
625	UUAAAUAU A UAUUCA	761	GGUAAAUAU U AGUAAU
627	AAAUAUAU A UUUCAGA	762	GUAAAUAU JU A GUAAUJA
629	AAUAAUJU U UCAGATA	765	AAUUAGU A UUUAAU
630	AUAAUAAU U CAGAUAU	767	UUAGUAU U UAUUAA
631	UAAJUUU C AGAAUAC	768	UAGUAUJU U AUUUAU
636	UUCAGAU A UCAGAAU	769	AGUADUU A UUUAUAG
638	CAGAUAU C AGAAUCA	771	UAAUUAU U UAAUGUU
644	UCAGAAU C AUUGAAG	772	AAUUAJU U AAUGUUA
647	GAACAUU U GAAGUAJ	773	UUUADUU A AUGUUAJ
653	UUGAAGU A UUUUCU	778	UUAADGU U ADGUUGU
655	GAAGUAU U UUCCUCC	779	UAADGUU A UGUUGUG
656	AAGUADU U UCCUCCA	783	GUUAJGU U GGUUCU
657	AGUAAJU U CCUOCAG	788	GUUGUGU U CUAUAA
658	GUAAJUU C CUCCAGG	789	UUGUGUU C UAAUAAA
661	UUUUCU C CAGGCAA	791	GUGUUCU A AUAAAAC
672	GCIAAAAU U GAUAAAC	794	UUCUAAU A AAACAAA
676	AAUUGAU A UACUUUU	805	CAAAAU A GACAACU
678	UUGAUAU A CUUUUU		
581	AUAAUACU U UUUUCUU		
682	UAUACUU U UUUCJUA		

Table 12: Human IL-5 HH Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
8	GCAAAGA CUGAUGAGGCCGAAAGGCCGAA AGUGCAU
9	GGCAAG CUGAUGAGGCCGAAAGGCCGAA AAGUGCA
10	UGGCAA CUGAUGAGGCCGAAAGGCCGAA AAAGUGC
12	UUUGGC CUGAUGAGGCCGAAAGGCCGAA AGAAAGU
13	CUUUGC CUGAUGAGGCCGAAAGGCCGAA AAGAAGG
36	GCUCUGA CUGAUGAGGCCGAAAGGCCGAA ACGUUCU
37	GGCUCUG CUGAUGAGGCCGAAAGGCCGAA AACGUUC
38	UGGCUCU CUGAUGAGGCCGAAAGGCCGAA AAACGUU
56	AADGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAUCC
57	AAAUGCA CUGAUGAGGCCGAAAGGCCGAA AAGCAUC
63	AAACUCA CUGAUGAGGCCGAAAGGCCGAA AUGCAGA
64	CAAACUC CUGAUGAGGCCGAAAGGCCGAA AAUGCAG
69	GCUAGCA CUGAUGAGGCCGAAAGGCCGAA ACUCUAA
70	AGCUAGC CUGAUGAGGCCGAAAGGCCGAA AACUCAA
74	CAAGAGC CUGAUGAGGCCGAAAGGCCGAA AGCIAAC
78	GCUCCAA CUGAUGAGGCCGAAAGGCCGAA AGCUAGC
80	CAGCUCC CUGAUGAGGCCGAAAGGCCGAA AGAGCTA
91	AUACACG CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
97	GAUGGCA CUGAUGAGGCCGAAAGGCCGAA ACACGUA
104	CUGUGGG CUGAUGAGGCCGAAAGGCCGAA AUGGCAU
116	UUGUGGG CUGAUGAGGCCGAAAGGCCGAA AUUUCUG
117	CUUGUGG CUGAUGAGGCCGAAAGGCCGAA AUUUUCU
130	UUUCACC CUGAUGAGGCCGAAAGGCCGAA AUGCACU
145	CAGUGCC CUGAUGAGGCCGAAAGGCCGAA AGGUUCU
155	GAGUAGA CUGAUGAGGCCGAAAGGCCGAA AGCAGUG
156	UGAGUAG CUGAUGAGGCCGAAAGGCCGAA AAGCAGU
157	AUGAGUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAG
159	CGAUGAG CUGAUGAGGCCGAAAGGCCGAA AGAAAGC
162	GUUCGAI CUGAUGAGGCCGAAAGGCCGAA AGUAGAA
165	AGAGUUC CUGAUGAGGCCGAAAGGCCGAA AUGAGUA
171	AUCAGCA CUGAUGAGGCCGAAAGGCCGAA AGUUCGA
179	CAUTGGC CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
192	AUCCUCA CUGAUGAGGCCGAAAGGCCGAA AGUCUCA
200	GAACAGG CUGAUGAGGCCGAAAGGCCGAA AUCCUCA
201	GGAAACAG CUGAUGAGGCCGAAAGGCCGAA AAUCCUC
206	GUACAGG CUGAUGAGGCCGAAAGGCCGAA ACAGGAA
207	UGUACAG CUGAUGAGGCCGAAAGGCCGAA AACAGGA
212	UUUUUAUG CUGAUGAGGCCGAAAGGCCGAA ACAGGAA
216	UGAUUUU CUGAUGAGGCCGAAAGGCCGAA AUGUACA
222	AGUUGGU CUGAUGAGGCCGAAAGGCCGAA AUUUUUA
245	CCUGAAA CUGAUGAGGCCGAAAGGCCGAA AUUUCUU

247 UCCCUGA CUGAUGAGGCCGAAAGGCCGAA AGAUUUC
 248 UUCCUG CUGAUGAGGCCGAAAGGCCGAA AAGAUUU
 249 AUUCCU CUGAUGAGGCCGAAAGGCCGAA AAAGAUU
 257 GUGGCC CUGAUGAGGCCGAAAGGCCGAA AUUCCU
 273 ACAGUU CUGAUGAGGCCGAAAGGCCGAA ACUCUCC
 291 UCCACAG CUGAUGAGGCCGAAAGGCCGAA ACCCCCCU
 305 UUUUGAA CUGAUGAGGCCGAAAGGCCGAA AGUCUUU
 307 GUUUUUG CUGAUGAGGCCGAAAGGCCGAA AUAGUCU
 308 AGUUUUU CUGAUGAGGCCGAAAGGCCGAA AAUAGUC
 316 UAAGGAC CUGAUGAGGCCGAAAGGCCGAA AGUUUUU
 319 UAUUAAG CUGAUGAGGCCGAAAGGCCGAA ACAAGUU
 322 CUUUAUU CUGAUGAGGCCGAAAGGCCGAA AGGACAA
 323 UCUUUAU CUGAUGAGGCCGAAAGGCCGAA AAGGACA
 326 AUUUCUU CUGAUGAGGCCGAAAGGCCGAA AUUAAGG
 334 GUCAADG CUGAUGAGGCCGAAAGGCCGAA AUUUCUU
 338 GGCGGUC CUGAUGAGGCCGAAAGGCCGAA AUGUAUU
 380 AUUGGUU CUGAUGAGGCCGAAAGGCCGAA ACUCUCC
 388 GUUAGG CUGAUGAGGCCGAAAGGCCGAA AUUUGGU
 389 AGUCUAG CUGAUGAGGCCGAAAGGCCGAA AAUUGGU
 392 GGUAGUC CUGAUGAGGCCGAAAGGCCGAA AGGAADU
 397 UUGCAGG CUGAUGAGGCCGAAAGGCCGAA AGUCUAG
 409 ACCAAGA CUGAUGAGGCCGAAAGGCCGAA ACUCUUG
 410 CACCAAG CUGAUGAGGCCGAAAGGCCGAA AACUCUU
 411 ACACCAA CUGAUGAGGCCGAAAGGCCGAA AAACUCU
 413 UUACACC CUGAUGAGGCCGAAAGGCCGAA AGAAACU
 419 UGUUCAU CUGAUGAGGCCGAAAGGCCGAA ACACCAA
 437 UUUCUAA CUGAUGAGGCCGAAAGGCCGAA AUCCACU
 440 AACUUUC CUGAUGAGGCCGAAAGGCCGAA AUUAUCC
 447 UAGUCUC CUGAUGAGGCCGAAAGGCCGAA ACUUUCU
 454 ACCAGUU CUGAUGAGGCCGAAAGGCCGAA AGUCUCA
 462 UGCAACA CUGAUGAGGCCGAAAGGCCGAA ACCAGUU
 463 CUGAAC CUGAUGAGGCCGAAAGGCCGAA AACCAAGU
 466 UGGCUGC CUGAUGAGGCCGAAAGGCCGAA ACAAAACC
 479 CCUCCAA CUGAUGAGGCCGAAAGGCCGAA AUCUUUG
 480 UCCUCCA CUGAUGAGGCCGAAAGGCCGAA AAUCUUU
 481 CUCCUCC CUGAUGAGGCCGAAAGGCCGAA AAAUCUU
 497 GCAGUAA CUGAUGAGGCCGAAAGGCCGAA AUGUCU
 498 UGCAGUA CUGAUGAGGCCGAAAGGCCGAA AAUGUCC
 499 CUGCAGU CUGAUGAGGCCGAAAGGCCGAA AAAUGUC
 500 ACUGGAG CUGAUGAGGCCGAAAGGCCGAA AAAADGU
 531 AAGGCCU CUGAUGAGGCCGAAAGGCCGAA ACUCUUU
 538 GAAAAAU CUGAUGAGGCCGAAAGGCCGAA AGGCCUG
 539 UGAAAAAU CUGAUGAGGCCGAAAGGCCGAA AAGGCCU
 542 UAUUGAA CUGAUGAGGCCGAAAGGCCGAA AUUAAGG
 543 AUAUUGA CUGAUGAGGCCGAAAGGCCGAA AAUUAAG
 544 UAUAUUG CUGAUGAGGCCGAAAGGCCGAA AAAUUA
 545 UUAUUAU CUGAUGAGGCCGAAAGGCCGAA AAAAUUA
 549 UAAAUAU CUGAUGAGGCCGAAAGGCCGAA AUUGAAA
 551 GUAAAUAU CUGAUGAGGCCGAAAGGCCGAA AUAUUGA

554 GAAGUUA CUGAUGAGGCCGAAAGGCCGAA AUUAUAU
 555 UGAAGUU CUGAUGAGGCCGAAAGGCCGAA AAUUAUA
 556 CUGAAGU CUGAUGAGGCCGAAAGGCCGAA AAAUUAU
 560 CCCUCUG CUGAUGAGGCCGAAAGGCCGAA AGUAAA
 561 UCCCUCU CUGAUGAGGCCGAAAGGCCGAA AAGUAAA
 573 AAAUAUU CUGAUGAGGCCGAAAGGCCGAA ACUUUCC
 577 CCUGAAA CUGAUGAGGCCGAAAGGCCGAA AUUUACU
 579 UGCCUGA CUGAUGAGGCCGAAAGGCCGAA AUAUUUA
 580 AUGCCUG CUGAUGAGGCCGAAAGGCCGAA AAUAUGU
 581 UAUGCCU CUGAUGAGGCCGAAAGGCCGAA AAAUAUU
 588 GUGUCAG CUGAUGAGGCCGAAAGGCCGAA AUGCCUG
 597 UCUGGCA CUGAUGAGGCCGAAAGGCCGAA AGUGUCA
 598 UUCUGGC CUGAUGAGGCCGAAAGGCCGAA AAGUGUC
 611 AGAAUUU CUGAUGAGGCCGAAAGGCCGAA AUGCUUU
 616 UUUUAAG CUGAUGAGGCCGAAAGGCCGAA AUUUUAU
 617 AUUUUAA CUGAUGAGGCCGAAAGGCCGAA AUUUUUA
 619 AUAUUUU CUGAUGAGGCCGAAAGGCCGAA AGAAUUU
 620 UAUAUJJ CUGAUGAGGCCGAAAGGCCGAA AAGAAUU
 625 UGAAAUA CUGAUGAGGCCGAAAGGCCGAA AUUUUAA
 627 UCUGAAA CUGAUGAGGCCGAAAGGCCGAA AUAUUUU
 629 UAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUUAUJJ
 630 AUAUJCUG CUGAUGAGGCCGAAAGGCCGAA AUUUAU
 631 GAAUUCU CUGAUGAGGCCGAAAGGCCGAA AAAUUA
 636 AUUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCUGAA
 638 UCAUUCU CUGAUGAGGCCGAAAGGCCGAA AUUJCUG
 644 CUUCAAU CUGAUGAGGCCGAAAGGCCGAA AUUCUGA
 647 AUACUUC CUGAUGAGGCCGAAAGGCCGAA AUGAUUC
 653 AGGAAAA CUGAUGAGGCCGAAAGGCCGAA ACUUCAA
 655 GGAGGAA CUGAUGAGGCCGAAAGGCCGAA AUACUUC
 656 UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AAUACUU
 657 CUGGAGG CUGAUGAGGCCGAAAGGCCGAA AAUACU
 658 CCUGGAG CUGAUGAGGCCGAAAGGCCGAA AAAUAC
 661 UUGCCUG CUGAUGAGGCCGAAAGGCCGAA AGGAAAA
 672 GUAAUAC CUGAUGAGGCCGAAAGGCCGAA AUUUUGC
 676 AAAAGUA CUGAUGAGGCCGAAAGGCCGAA AUCAAUU
 678 AAAAAG CUGAUGAGGCCGAAAGGCCGAA AUAUCAA
 681 AAGAAAA CUGAUGAGGCCGAAAGGCCGAA AGUAUAU
 682 UAAGAAA CUGAUGAGGCCGAAAGGCCGAA AAGUADA
 683 AUAGAAA CUGAUGAGGCCGAAAGGCCGAA AAAGUAU
 684 AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAAAGUA
 685 AAAUAG CUGAUGAGGCCGAAAGGCCGAA AAAAGU
 686 UAAAUAA CUGAUGAGGCCGAAAGGCCGAA AAAAAG
 688 GUAAAUA CUGAUGAGGCCGAAAGGCCGAA AGAAAAAA
 689 AGUAAA CUGAUGAGGCCGAAAGGCCGAA AAGAAAAA
 691 UAAGUUA CUGAUGAGGCCGAAAGGCCGAA AUAAAGAA
 692 UUAAGUU CUGAUGAGGCCGAAAGGCCGAA AAUAAGA
 693 GUUAAGU CUGAUGAGGCCGAAAGGCCGAA AAAUAAG
 697 GAAUGUU CUGAUGAGGCCGAAAGGCCGAA AGUAAA
 698 AGAAUGU CUGAUGAGGCCGAAAGGCCGAA AAGUUA

703	UUUACAG CUGAUGAGGCCGAAAGGCCGAA AUGUUA
704	UUUUACA CUGAUGAGGCCGAAAGGCCGAA AAUGUUA
708	GACAUUU CUGAUGAGGCCGAAAGGCCGAA ACAGAAU
715	GUUAACA CUGAUGAGGCCGAAAGGCCGAA ACAUUUU
719	UUAAGUU CUGAUGAGGCCGAAAGGCCGAA ACAGACA
720	AUUAAGU CUGAUGAGGCCGAAAGGCCGAA AACAGAC
724	UACUAUU CUGAUGAGGCCGAAAGGCCGAA AGUUAAC
725	AUACUAU CUGAUGAGGCCGAAAGGCCGAA AAGUUA
728	UAAAACAC CUGAUGAGGCCGAAAGGCCGAA AUUAAGU
731	UCAUAAA CUGAUGAGGCCGAAAGGCCGAA ACUAUUA
733	UUUCAUU CUGAUGAGGCCGAAAGGCCGAA AUACUAU
734	AGCUCAU CUGAUGAGGCCGAAAGGCCGAA AAUACUA
735	CAUUUCA CUGAUGAGGCCGAAAGGCCGAA AAUACU
745	AAUUCUU CUGAUGAGGCCGAAAGGCCGAA ACCAUUU
746	AAAUUCU CUGAUGAGGCCGAAAGGCCGAA AACCAUU
752	UUUACCA CUGAUGAGGCCGAAAGGCCGAA AUUCUUA
753	AUUUACC CUGAUGAGGCCGAAAGGCCGAA AAUUCUU
757	ACUAAUU CUGAUGAGGCCGAAAGGCCGAA ACCAAAU
761	AAUACU CUGAUGAGGCCGAAAGGCCGAA AUUUACC
762	UAAAACAC CUGAUGAGGCCGAAAGGCCGAA AAUUAC
765	AAUAAA CUGAUGAGGCCGAAAGGCCGAA ACUAAIU
767	UAAAAUA CUGAUGAGGCCGAAAGGCCGAA AUACUAA
768	AUAAAUAU CUGAUGAGGCCGAAAGGCCGAA AAUACUA
769	CAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUACU
771	AACAUUA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
772	UAAACAUU CUGAUGAGGCCGAAAGGCCGAA AAUAAA
773	AUAACAU CUGAUGAGGCCGAAAGGCCGAA AAAUAAA
778	ACAACAU CUGAUGAGGCCGAAAGGCCGAA ACAUUA
779	CACAAAC CUGAUGAGGCCGAAAGGCCGAA AACAUUA
783	AGAACAC CUGAUGAGGCCGAAAGGCCGAA ACAUAC
788	UUAUJAG CUGAUGAGGCCGAAAGGCCGAA ACACAC
789	UUUAAUA CUGAUGAGGCCGAAAGGCCGAA AACACAA
791	GUUUUAU CUGAUGAGGCCGAAAGGCCGAA AGAACAC
794	UUUGUUU CUGAUGAGGCCGAAAGGCCGAA AUUAGAA
805	AGUUGUC CUGAUGAGGCCGAAAGGCCGAA AUUUUUG

Table 13: Mouse IL-5 HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	cGGcuCUU c CUUUGCu	253	AGGGgcU A GaCAuAC
11	uCuuCCU U UGQugAA	259	UagACAU a CUGaAgA
12	CUUCCUU U GCugAAG	269	GaAGAaU C AAAACUGU
36	GAAGacU U CAGAGGuC	269	GaAGAaU c AAAACugU
36	GaAgAcU u cAgAGUc	269	GAAgaAU c sAAcUgU
37	AAGacJU C AGAGuCA	287	uGGGGGU A CUGUGGA
43	UcaGaGU c AUGAgAA	301	AAAUGCU A UUCcAAA
58	GGAUgCU U CUGCACU	301	AAAUGCU a uUCCaaA
59	GAUgCUU C UGCACUU	303	AUGCuAU u CCaAaAc
59	gAUgCUU c uGcAcUU	303	AugCUAU U CcAAAAC
66	CUGCACU U GAGUgUu	304	uGCUAUU C CAAAACC
82	UgAcucU c agcUGUG	315	AAcCUGU C aUUAUA
91	GcUgUGU c uggGCCA	318	cUGUCAU U AAUAAAG
112	ugGAgAU U CCCAugA	319	UGUCAUU A ATAAAGA
113	gGAgAUU C CCAugAG	322	CaUUAAU A AAGAAAU
141	GAGACCU U GaCACaG	330	AAgAAAU A CAUTGAC
141	GAgACcU U GaCACAcG	334	AAUACAU U GACCGCC
158	gUCcgCU C AcCGAgC	334	AAUaCau u GACcgCC
167	ccGAgCU C UGUUGAc	384	AggCAGU U CCUgGAu
196	UGAGGcU U CCUGUcC	385	ggCAGUU C CUgGAuU
197	GAGGcUU C CUGUcCC	393	CUGGAuU A CCUGCAA
197	gAGGQuU c CUGUCCc	405	CAAGAGU U CCUJGGU
202	UUCCUGU c CCUacuC	406	AAAGAGUU c CUUGGUG
202	UUCCUGU c CcUAcuc	409	AGUUccU U GGUGUgA
206	UGUCCcU a cuCaUAA	481	UcaCAAU u UAAGJUA
212	UACUCAU a AAAaUCA	482	cACAAUU U AAgUUaA
212	UacuCAU A AAAAUCA	483	ACAAUUU A AgUUaAa
218	UaaAaaU c acCcAGCU	483	ACAAUuU a aGUUAAA
218	UAAAAAAU C ACCAgCU	495	AAAUUgU c AACAgAU
218	uAAAAAAU c acCAGCU	553	GCTGUuU c CaUuUAU
232	uaUGCAU U GGAGAAA	557	UuUcCAU U UauaUUU
241	gAGAAAaU C UUUCAGG	564	UUauAuU u aUgUCCU
241	gAgAaAU c UUucAGG	564	UUauaUU u AugUccU
241	gagAAAU c UUUCAGG	565	uaUAUUU a ugUCQuG
241	gAgAaAU c UUUCAGg	565	UUAUaUUG a UgUCcUg
243	gaAAuU U UCAGgGg	569	UUUaUUGU c cUGUaGU
243	GAAAUCU U UCAGGGg	569	uUUAUgU c cUGUaGU
244	AAAUCUU U CAGGGgc	613	AAAGUgU u uaACCUU
245	AAUCUUU C AGGGgcU	614	AAgUGUu U eACcUUU

620	UUAACcU u uUuGUAU	1407	cCAgUUU A CUcCAGG
793	caAGgCU u UGuGcAU	1407	ccAgUUU a CUCCAGG
816	CUGagUU a UACUCcc	1410	gUUUAcU C CAGGaaAA
818	GAgUUAU a cUCCcuC	1434	AUgCUUU U aUuUaAU
825	ACUcCcU c CccCUCA	1434	aUgcUuU U AUUUAAu
825	aCUccCU c CcCcUCA	1434	aUgcuUU u AuUUAAU
839	AuCucU U CGUUGC	1435	UgCUUUU a UuUaAUU
840	uCucUU c GUUGCAu	1435	ugcUUUU a uUUAAuU
863	cAAGUAU U cCAGGCu	1438	UuUUAAU U AAuUcug
864	AAgUAUU c CAGGCug	1438	uUUUAAU U AAUucUg
864	AAGUAUU c caggCug	1439	UUUAAUU A AUucUgU
913	gAaCUCU U GGucCaG	1443	UUUaAuU c UGuaAGa
917	UcUuggU c CAGAUuGG	1447	AUUCUGU A AgAUGUu
957	UUagcAU c CUUUCUc	1458	ugUUcaU a UUAAUUA
960	GCAuccU u UcUcCuA	1458	ugUUcAU A uUAAUUA
960	GcaUcCU u uCUCCUa	1460	UucAUAU u AUUUAug
962	AUcCuuU c UCCuUaGC	1461	UcAUAUU A UUUAAUG
975	gcccCUU u AgAUAGA	1463	AUAUoAU U UAUGAug
987	aGaUGAU A cuuAAUG	1475	AuGgAUU c aGUAAgU
990	UGAUACU u AAugacU	1479	AUUcaGU A AgUUAaU
1000	UGACuCU c UugCuGA	1483	aGuAAGU u AAUAAUJ
1027	CgggGCU U cCUGcUC	1483	aGUAAgU U AaUAAUJ
1034	UCCUGcU C CUaUcuA	1484	GUAAgUU A aUAUUA
1037	UgeUCCu A UcUAACU	1487	agUUAAU a UUuAAUA
1039	cUccuAU c UAACUUC	1487	AgUUAaU A UGUAAUa
1039	cUCCUAU c UAACUUC	1489	UUAAUdAU U uAuUAcA
1041	CcUAUcU A ACUUCaa	1489	UUAAwAU u UAUuaCA
1051	UUCAAuU U AAuAccC	1489	UUAAuAU U UAUuAcA
1148	uGAcUUU u cUuaUGU	1490	UAUUaUU u AuUAcAc
1213	GCUGGaU u UGGAAA	1490	UAaUAUU U AUuAcAc
1213	gcUGGAU u uGgAAA	1490	UAaUAUU U AUUAcAc
1214	cugGAUU U UGGAAA	1491	AAUAAUU a uuaAcG
1215	ugGAUUU U GGAaaAG	1491	AAUAUuU a UuAcAcG
1234	gGGACAU c UccuUGC	1491	AaUAUUU A UuAcAcG
1236	GACAUcU c cuUGCAG	1491	AaUAUUU A UJAcAcG
1275	ugGGCCU U AcUUcUC	1494	AUuUAUU a CAcgUAU
1276	gGGCCUU A cuUcUCC	1502	cACGUaU A UaaAUu
1280	CUUAcUU c UCcgUgU	1502	cAcGUAU a UAAUaUU
1298	UgAACUU a AGAaGcA	1507	AUAUAaU a UUcUaaU
1310	gCAAAGU a aaUACCa	1509	AUAAuAU U CUaAuAA
1310	GCAAAGU a aAUAcca	1509	aUzaUaU U CUAAUAA
1310	GcaAAgU a AAUAccA	1510	UAAuAUU C UaAuAAa
1350	AAAGCAU A AAAUggU	1510	UAAuAUU C UaaauAAA
1358	AAAUGGU U ggGAugU	1510	UAAuAUU C UaaUAAA
1370	UgUuaUU C AGgUAUC	1510	UaaUaUU C UAAUAAA
1375	UUCAGgU A UCAGggU	1512	aUaUUUCU A AUAAAaC
1377	CAGgUAU C AGggUCA		UUCUAAU A AAgCAgA
1383	UCAGggU C AcUGgAG		
1405	cccCAgU U UACUcCA		

Table 14: Human IL-6 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
86	UACAGUA AGAA GCGCA ACCAGAGAACACCGUUGGGUACAUUACCUGUA	UCCGCCU GCC UACGUGUA
151	GAGUAGAA AGAA GCGCA ACCAGAGAACACCGUUGGGUACAUUACCUGUA	UCCGCCU GCU UCCUCACU
172	UCCGUAC AGAA GCGUC ACCAGAGAACACCGUUGGGUACAUUACCUGUA	GAACCU GCU GAAAGCCA
203	UGUACAGG AGAA GCGAU ACCAGAGAACACCGUUGGGUACAUUACCUGUA	GAUCCU GCU CCUGUACA

Table 15: Mouse IL-5 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
75	ACCUUGAGA AGAA GACAC ACCAGAGAACACACGGGUCACAUUACCGGA	GUGUCU GAC UCUCCU
83	CCAGACAC AGAA GAGGU ACCAGAGAACACACGGGUCACAUUACCGGA	ACUCUA GCU GUCCUC
147	GAGGGAC AGAA GUGUCA ACCAGAGAACACACGGGUCACAUUACCGGA	UGACACA GCU GUCCUC
150	GGUGAGG AGAA GUGUG ACCAGAGAACACACGGGUCACAUUACCGGA	CACAGCU GCU GCGUAC
154	CCUCGCG AGAA GACGC ACCAGAGAACACACGGGUCACAUUACCGGA	CCUGUC GCU CACGAGC
168	UCCUCGUC AGAA GGGUC ACCAGAGAACACACGGGUCACAUUACCGGA	GGCCUC GCU GACAGGC
199	UGAGGAGG AGAA GGAGC ACCAGAGAACACACGGGUCACAUUACCGGA	CCUUCU GUC OCACUCA
274	CCCCACG AGAA GGUUG ACCAGAGAACACACGGGUCACAUUACCGGA	UCUACU GUC CGGGGG
381	AUCCPAGG AGAA GGUUG ACCAGAGAACACACGGGUCACAUUACCGGA	CGAGCA GUU CCUGGAGU
454	CACCAUGG AGAA GGUUG ACCAGAGAACACACGGGUCACAUUACCGGA	CUGACU GCU CCUGGUG
499	GUUUUGC AGAA GUUGAC ACCAGAGAACACACGGGUCACAUUACCGGA	GUCAAC GAU GCAAAAC
548	UAAAUGGA AGAA GCAAU ACCAGAGAACACACGGGUCACAUUACCGGA	AUUCGU GUU UCCAUUA
701	GTAGGAGG AGAA GAAAU ACCAGAGAACACACGGGUCACAUUACCGGA	AAUUCU GAU CCUCUC
710	GTAGGAGA AGAA GGAGG ACCAGAGAACACACGGGUCACAUUACCGGA	UCCUCU GCU UCCUCUC
870	AGUCUAAA AGAA GCGUGG ACCAGAGAACACACGGGUCACAUUACCGGA	CCAGGU GAC UGCGACU
919	CUGCGGCC AGAA GGACCA ACCAGAGAACACACGGGUCACAUUACCGGA	UGGUCA GAU GGACCGAG
1030	UAGAUATGG AGAA GGAAGC ACCAGAGAACACACGGGUCACAUUACCGGA	GCUUCU GCU CCUBUCUA
1170	AUGCCACA AGAA GGUUC ACCAGAGAACACACGGGUCACAUUACCGGA	UGAUUCU GAC UGUGCU
1205	CAAAUCC AGAA GCGCCA ACCAGAGAACACACGGGUCACAUUACCGGA	UGGAGCA GCU GGAUUUG
1402	CUGGAGCA AGAA GGGGA ACCAGAGAACACACGGGUCACAUUACCGGA	UCCCGCA GUU UACUCCAG
1421	MAGCAUAC AGAA GGUUU ACCAGAGAACACACGGGUCACAUUACCGGA	AAAAACA GAU GUAUGCU

Table 16 : Mouse II-5 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
75	ACCUAGCA AGAA GAAAC ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	GUUCU GAC UCCAUU
83	CCUAGCUC AGAA GAGGU ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	ACUCA GCU GUUCUUG
147	GCGGCGAC AGAA GUGCA ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	UGACACA GCU GUUCUUC
150	GGCGAGCC AGAA GCGUG ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	CACGU GUC CCUCUCC
154	GCUCGGCG AGAA GACGCC ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	GGUGUC GCU CACGAGC
168	UGCTUGUC AGAA GNCUC ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	GACGUU GUU GACAGCA
199	UGAQUAGG AGAA GCAAGC ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	GUUCU GUC CCTACUCA
274	CCCCACCG AGAA GGUCA ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	UCAACU GUC CGUCGGG
381	AUCCOPCG AGAA GCTUC ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	CGGGCA GUU CGCGAUU
454	CACTUCCG AGAA GCUUG ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	CUGAGCU GCU CGAUGUG
499	GUUULLUGC AGAA GUUGC ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	GUCGACA GAU CGAAAAC
548	UAAUUGGA AGAA GCAUAU ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	AUACU GGU UCCUUA
701	CGAGGAGG AGAA GAAAU ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	AAUUCU GAU CCUCUC
710	GAAGGAGG AGAA GGAGGA ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	UCCUCU GGC UCCUCU
870	AGUCUAAA AGAA GCGUG ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	CGAGGU GAC UUUGACU
919	CUGGUCC AGAA GGACCA ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	UGGUCCA GAU GGAGCG
1030	UUGGUUAGG AGAA GGAAGC ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	GUUCU GCU CCUACUA
1170	AUGGCGCA AGAA GAUCA ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	UGAUCA GAC UGCGCAU
1205	CAAAUCC AGAA CCUCCA ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	UGGAGCA GCU GGAUUG
1402	CTTGTGCA AGAA CGGGCA ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	UCCCGA GGU UGCGCG
1421	ANCCAUUC AGAA GUUUU ACCAGAGAAACACACGUUUCGUUACAUUACUGUA	AAAACA GAU GUUCGUU

Table 17

Mouse *relA* HH Target sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
19	AAUGGCU a caCaGgA	467	cCAGGCCU c cuguUCg
22	aGCUCCu a cGJgGUG	469	AaGCCAU u AGccCAGC
26	CcUCcaU u GcGgACa	473	UuUgAGU C AGauCAg
93	GAuCUGU U uCCCCUc	481	AGCGaAU C CAGACCA
94	AuCUGUU u CCCCCUCA	501	AACCCCU U uCACGUU
100	UuCCCCU C AUCUUUc	502	ACCCCCU U CACGUUC
103	CCCUCAU C UUUuCCcU	508	UuCAGGU U CCTAUATAG
105	CUCAUU U uCCCUuCA	509	uCACGUU C CUAUAGA
106	UCAUCUU U CCCuCAG	512	cGUUCCU A UAGAgGA
129	CAGGCuU C UGGgCCU	514	UUCCUAU A GAGGAGC
138	GGgCCUu A UGUGGAG	534	GGGGACU A uGAcuUG
148	UGGAGAU C AUcGAac	556	UGOGccU C UGCUUCC
151	AGAUCAU c GAacAGC	561	cJCtGCU U CCAGGUG
180	AUGCGAU U CCGCUAU	562	UCDGCUU C CAGGUGA
181	UGCGaUU C CGCUAUa	585	aAGCCAU u AGccCAGc
186	UUCGGCU A uAAaUGC	598	GGCCCU C CuCCUga
204	GGGOGCU C aGOGGGC	613	CcCCUGU C CUCuCaC
217	GCAGUAU U CCUGGGCG	616	CUGUCCU c uCaCAUC
239	CACAGAU A CCACCAA	617	guCCUU C CUCAgCC
262	CCACCAU C AAGAUCA	620	CCUUCUU C AgCCaug
268	UCAAGAU C AAUGGCU	623	UCCUgcU u CCATCUC
276	AAUGGCU A CACAGGA	628	AUCCgAU u UUUGAUa
301	UuCGaAU C UCCCCUGG	630	CCgAUuU U UGAuAAC
303	CGaAUCU C CCUGGUC	631	CgAUUuU U GauAACc
310	CCCUGGU C ACCAAGG	638	UGgCcAU u GUGuUCC
323	GGccccU C CUCCuga	661	CCGAGCU C AACAUU
326	uCCaACCu C ACCGGCC	667	UCAAGAU C UGCGGAG
335	CCGGCCU C AuCCaCA	687	CGgAACU C UGGgAGC
349	AuGAacU U GUGGGgA	700	GCUGCCU C GGUGGGG
352	AGaUcaU c GaAcAGc	715	AUGAGAU C UUCCuUgC
375	GAUGGCU a CUAUAGAG	717	GAGAUU U CuUgCUG
376	AUGGUCU C UccGgaG	718	AGAUUU C uUgCUGU
378	GGCUaCU A UGAGGCCU	721	UucUCCU c CaUUGCG
391	CUGAccU C UGCCCaG	751	AaGACAU U GAGGUGU
409	GCaGuAU C CAuAGcU	759	GAGGUGU A UUUCACG
416	CCgCAGU a UCCAUAg	761	GGUGUAU U UCACGGG
417	CAuAGcU U CCAGAAC	762	GUGUAUU U CAOGGGG
418	AuAGcUU C CAGAACC	763	UGUAIUU C ACGGGAC
433	UGGGgAU C CAGUGUG	792	CGAGGCCU C CUUUUcu
795	GGCUCCU U UUCUCAA	1167	GAUGAGU U UuCCccc
796	GTUCCUU U UCUCAAG	1168	AUGAGUU U uCCeCCA
797	CUCCUUU U CuCAAGC	1169	UGAGUUU u CCcCCAU
798	UCCUUUU C uCAAGCU	1182	AUGcUGU U aCCaUCA
829	UGGOCAU U GUGUUCC	1183	UGcUGUU a CCaUCaG

834	AUUGUGU U CGGGACU	1184	GGccccU C CUcCUGa
835	UUGUGUU C CGGACUC	1187	GUccCUU c CUcaGCc
845	GACUCCU C CgUACGC	1188	UUaCCaU C aGGGCAG
849	CCUCCgU A OGCCGAC	1198	GGgAGUu u AGuCuGa
872	cCAGGCCU C CUGUuCG	1209	CAGGCCU a caCCUUC
883	UuCGaGU C UCCAUGC	1215	cuGGCCU U aGCacCG
885	CGaGUCU C CAUGCAG	1229	GGuCCCU u CCucAGc
905	GCGGCCU U CuGAuCG	1237	CCCAGcU C CUGCCCC
906	CGGCCUU C uGAuCGc	1250	CCAGGCCU C CAGgCuC
919	GcGAGCU C AGUGAGC	1268	CCCAGCU C CuGCCcc
936	AUGGA <u>g</u> U U CCAGUAC	1279	CCAUGGU c ccuucCU
937	UGGA <u>g</u> UU C CAGUACU	1281	gUGGgcU C AGCUgcG
942	UCCAGU A CuUGCCA	1286	AUgAGUu u UccCCCA
953	GCCuicAU c CACauGA	1309	CuCCUGU u CgAGUQu
962	AGAuGAU C GcCACCG	1315	ccccAGU u CUAaCCC
965	CagUacU u gCCaGAc	1318	CAGUuCU A aCCCCgG
973	ACCGGAU U GlaGAGA	1331	gGGUCCU C CcCAGuC
986	GAgACCu u cAA <u>g</u> agu	1334	CuuUUCU C AaGCUga
996	AGGACCU A UGAGACC	1389	ACGCUGU C gGAaGCC
1005	GAGACCU U CAAGAGU	1413	CUGCAGU U UGAUGcU
1006	AGACCUU C AAGAGUA	1414	UGCAGUU U GAUGcUG
1015	AGAGAUU C AUGAAGA	1437	GGGGCCU U GCUUGGC
1028	GAAGAGU C CUUUCUa	1441	CCUUGCU U GGCAACA
1031	GAGUCCU U UCAauGG	1467	GgaGUGU U CACAGAC
1032	AGUCCUU U CaauGGA	1468	gaGUGUU C ACAGACC
1033	GUCCUUU C AauGGAC	1482	CUGGCAU C uGUgGAC
1058	COGGCCU C CAAcCcCG	1486	CuUCgGU a GggAACU
1064	UaCACCU u GAuCCAA	1494	GACAACU C aGAGUUU
1072	GgCGuAU U CCUGUGC	1500	UCaGAGU U UCAGCAG
1082	UGUGCCU a COOGaAa	1501	CaGAGUU U CAGCAGC
1083	aaGOCUU C CCGaAGu	1502	agAGUUU C AGCAGCU
1092	CGaAaCU C AaCUUCU	1525	gGuGCAU C CCUGUGu
1097	CUChacU U CUGUCOC	1566	AUGGAGU A CCCUGAa
1098	UCAACUU C UGUCCCC	1577	UGAaGCU A UAACUCG
1102	CUUCUGU C CCCAAGC	1579	AaGCUAU A ACUCGCC
1125	CAGCCCCU A caccUUC	1583	UAIaAACU C GCCUgGU
1127	GCCaUAU a gCcUUAC	1588	CUUCCU A GaGAggG
1131	CAUCCCU c agCacCA	1622	CCCAGCU C CUGCcCC
1132	AcaCCUU c cCagCAU	1628	UCCUGCU u CggUaGG
1133	UCCaUcU c CagQuUC	1648	GGGGCCU u CCCAAUG
1137	UUUACQuU u AgCgCgc	1660	cUGaCCU C ugcccAG
1140	cCagCAU C CCUCAGC	1663	cuCUGCU U ccAGGUg
1153	GCACCAU C AACUuUG	1664	uCUGCUU c CAGGUgA
1158	AUCAACU u UGAUGAG	1665	CUCgcUU u CGGAGgU
1680	GAAGACU U CUCCUCC		
1681	AAGACUU C UCCUCCA		
1683	GACUUCU C CUCCAUU		
1686	UUCUCCU C CAUUGCG		
1690	CCUCCAU U GOGGACA		

1704	AUGGACU	U	CUCuGCU
1705	UGGACUU	C	U <u>C</u> GUUC
1707	GACUUCU	C	uGU <u>C</u> U
1721	uuUGAGU	C	AGA <u>U</u> CAG
1726	GUCAGAU	C	AGCUCCU
1731	AUCAGCU	C	CUAAGGU
1734	AGCUCCU	A	AGGuGcU
1754	CaGugCU	C	CCaAGAG

Table 18
 Human *rel A* HH Target Sequences
 nt. Position HH Target Sequence nt. Position HH Target Sequence

19	AAUGGCU C GUCUGUA	467	GCAGGCU A UCAGUCA
22	GGCUUGU C UGUAGUG	469	AGGCUAU C AGUCAGC
26	CGUCUGU A GUGCACG	473	UAUCAGU C AGCGCAU
93	GAACUGU U CCCCCUC	481	AGGCCAU C CAGACCA
94	AACUGUU C CCCCCUCA	501	AACCCCU U CCAAGUU
100	UCCCCCU C AUUUUCC	502	ACCCCTU C CAAGUCC
103	CCCUCAU C UUCCCOGG	508	UCCAAGU U CCUAUAG
105	CUCAUU C UCOGGCA	509	CCAAGUU C CUAUAGA
106	UCAUCUU C CGGGCAG	512	AGUUCCU A UAGAAGA
129	CAGGCCU C UGGGCCCC	514	UUCCUAU A GAAGAGC
138	GGCCCCU A UGUGGAG	534	GGGGACU A CGACCUG
148	UGGAGAU C AUUGAGC	556	UGCGGCU C UGCJUCC
151	AGAUCAU U GAGCAGC	561	CUCUGCU U CCAGGUG
180	AUGGCCU U CGCCUAC	562	UCUGCUU C CAGGUGA
181	UGCGCUU C CGCUACA	585	GACCCAU C AGGCAGG
186	UUCGGCU A CAAGUGC	598	GGCCCCU C CGCTUGC
204	GGGCGCU C CGCGGGC	613	CGCCUGU C CUUCCUC
217	GCAGCAU C CCAGCG	616	CUGUCCU U CCUCAUC
239	CACAGAU A CCACCAA	617	UGUCCUU C CUCAUCC
262	CCACCAU C AAGAUCA	620	CCUUCCU C AUCCCAU
268	UCAAGAU C AAUGGU	623	UCCUCAU C CCAUCUU
276	AAUGGCU A CACAGGA	628	AUCCCAU C UUUGACA
301	UGCGCAU C UCCCCUGG	630	CCCAUCU U UGACAAU
303	CGCAUCU C CCUGGUC	631	CCAUCUU U GACAAUC
310	CCCUGGU C ACCAAGG	638	UGACAAU C GUGCCCC
323	GGACCCU C CUCACCG	661	CCGAGCU C AAGAUCA
326	CCCUCUU C ACCGGCC	667	UCAAGAU C UGCGGAG
335	CGGGCCU C ACCCCCCA	687	CGAACU C UGGCAGC
349	ACGAGCU U GUAGGAA	700	GTUGOCU C GGUGGGG
352	AGCUUGU A GGAAAGG	715	AUGAGAU C UUCCUAC
375	GAUGGCU U CUAUGAG	717	GAGAUCU U CCUACUG
376	AUGGCCU C UAUAGAG	718	AGAUUU C CUACUGU
378	GGCUUCU A UGAGGCU	721	UCUUCU A CUGUGUG
391	CUGAGCU C UGCCCGG	751	AGGACAU U GAGGUGU
409	GCUGCAU C CACAGUU	759	GAGGUGU A UUUCACG
416	CCACAGU U UCCAGAA	761	GGUGUAU U UCACGGG
417	CACAGUU U CCAGAAC	762	GUGUAUU U CAAGGGA
418	ACAGUUU C CAGAAC	763	UGUAUUU C ACGGGAC
433	UGGGAAU C CAGUGUG	792	CGAGGCU C CUUUUCG
795	GGCUCCU U UUOGCAA	1167	GAUGAGU U UCCCACC
796	GCUCUUU U UCGCAAG	1168	AUGAGUU U CCCACCA
797	CUCCUUU U CGCAAGC	1169	UGAGUUU C CCACCAU
798	UCCUUUU C GCAAGCU	1182	AUGGUGU U UCCUUCU
829	UGGCCAU U GUGUUCC	1183	UGGUGUU U CCUUCUG
834	AUUGUGU U CGGGACC	1184	GGUGUUU C CUUCUGG

835	UUGUGUU C CGGACCC	1187	GUUUCCU U CUGGGCA
845	GACCCCCU C CCTAACGC	1188	UUUCCUU C UGGGCAG
849	CCUCCCU A CGCAGAC	1198	GGCAGAU C AGCCAGG
872	GCAGGCCU C CUGUGCG	1209	CAGGCCU C GGCCUUG
883	UGCGGUGU C UCCAUUGC	1215	UCGGCCU U GGCCCCG
885	CGUGUCU C CAUGCAG	1229	GGCCCCU C CCCAAGU
905	GCGGCCU U CCGACCG	1237	CCCAGU C CUGCCCC
906	CGGCCUU C CGACCGG	1250	CCAGGU C CAGCCCC
919	GGGAGGU C AGUGAGC	1268	CCUCGU C CAGCCAU
936	AUGGAAU U CCAGUAC	1279	CCAUGGU A UCAGCUC
937	UGGAAUU C CAGUACC	1281	AUGGUAU C AGCUCUG
942	UUCAGU A CCUGCCA	1286	AUCAGGU C UGGGCCA
953	GCCAGAU A CAGACGA	1309	CCCCUGU C CCAGUCC
962	ACACGAU C GUCACCG	1315	UCCAGU C CUAGCCC
965	CGAUUGU C ACOGGAU	1318	CAGUCCU A GCCCCAG
973	ACCGGUU U GAGGAGA	1331	AGGCCCCU C CUCAGGC
986	GAAACGU A AAAGGAC	1334	CCUCUCCU C AGGCUGU
996	AGGACAU A UGAGACC	1389	ACGCUGU C AGAGGCC
1005	GAGACCU U CAAGAGC	1413	CUGGAGU U UGAUGAU
1006	AGACCUU C AAGAGCA	1414	UGCAGUU U GAUGAUG
1015	AGAGCAU C AUGAAGA	1437	GGGGCCU U GCUUGGC
1028	GAAGAGU C CUUUCAG	1441	CCUUGGU U GGCAACA
1031	GAGUCCU U UCAGCGG	1467	GCUGUGU U CACAGAC
1032	AGUCCUU U CAGOGGA	1468	CUGUGUU C ACAGACC
1033	GUCCUUU C AGCGGAC	1482	CUGGCAU C CGUOGAC
1058	CGGGCCU C CACUCCG	1486	CAUCCGU C GACAACU
1064	UCCACCU C GACGGAU	1494	GACAAUCU C CGAGUUU
1072	GACGCAU U GCUGUGC	1500	UCOGAGU U UCAGCAG
1082	UGUGGCCU U CCGCAG	1501	CCGAGUU U CAGCAGC
1083	GUGCCUU C CCGCAGC	1502	CGAGUUU C AGCAGCU
1092	CGCAGGU C AGCUUUC	1525	AGGGCAU A CCUGUGG
1097	CUCAGGU U CUGUOCC	1566	AUGGAGU A CCCUGAG
1098	UCAGGUU C UGUCCCC	1577	UGAGGCU A UAACUCG
1102	CUUCUGU C CCCAAGC	1579	AGGCUAU A ACUCGCC
1125	CAGGCCU A UCCCCUU	1583	UAUAACU C GCCUAGU
1127	GCCCCAU C CCUUUAC	1588	CUCGCCU A GUGACAG
1131	UAUCCCU U UACGUCA	1622	CCCAGCU C CUGGUCC
1132	AUCCGUU U ACGUCAU	1628	UCCUGGU C CACUGGG
1133	UCCCCUU A CGUCAUC	1648	CGGGGCCU C CCCAAUG
1137	UUUACGU C AUCCCCG	1660	AUGGGCCU C CUUUCAG
1140	ACGUCAU C CCUGAGC	1663	GCCUCCU U UCAGGAG
1153	GCACCAU C AACUAUG	1664	CCUOCUU U CAGGAGA
1158	AUCAACU A UGAUGAG	1665	CUCCUUU C AGGAGAU
1680	GAAGACU U CUCCUOC		
1681	AAGACUU C UCCUCCA		
1683	GACUUCU C CUCCAUU		
1686	UUCUCCU C CAUUGCG		
1690	CCUOCAU U GCGGACA		
1704	AUGGACU U CUCAGCC		

1705	UCCACUU C UCAGCCC
1707	GACUUCU C AGCCCCUG
1721	GCUGAGU C AGAUCAAG
1726	GUCAAGAU C AGCUCCU
1731	AUCAGCU C CUAAGGG
1734	AGCUCCU A AGGGGGU
1754	CUGCCCCU C CCCAGAG

Table 19

Mouse *rel A* HH Ribozyme Sequences

nt.	HH Ribozyme Sequence
Sequence	

19	UCCUGUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
22	CACCAAG CUGAUGAGGCCGAAAGGCCGAA AGGAGCU
26	UGUCCGC CUGAUGAGGCCGAAAGGCCGAA AUGGAGG
93	GAGGGGA CUGAUGAGGCCGAAAGGCCGAA ACAGAUC
94	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA AACAGAU
100	GAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGGGAA
103	AGGGAAA CUGAUGAGGCCGAAAGGCCGAA AUGAGGG
105	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA AGAUGAG
106	CUGAGGG CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
129	AGGCCCA CUGAUGAGGCCGAAAGGCCGAA AAGCCUG
138	CUCCACA CUGAUGAGGCCGAAAGGCCGAA AAGGCC
148	GUUCGAU CUGAUGAGGCCGAAAGGCCGAA AUCUCCA
151	GCUGUUC CUGAUGAGGCCGAAAGGCCGAA AUGAUCU
180	AUAGCGG CUGAUGAGGCCGAAAGGCCGAA AUCGCAU
181	UAUAGCG CUGAUGAGGCCGAAAGGCCGAA AAUCGCA
186	GCAUUUA CUGAUGAGGCCGAAAGGCCGAA AGCGGAA
204	GCCCCGU CUGAUGAGGCCGAAAGGCCGAA AGCGCCC
217	CGCCAGG CUGAUGAGGCCGAAAGGCCGAA AUACUGC
239	UUGGUGG CUGAUGAGGCCGAAAGGCCGAA AUCUGUG
262	UGAUCUU CUGAUGAGGCCGAAAGGCCGAA AUGGUGG
268	AGCCAUU CUGAUGAGGCCGAAAGGCCGAA AUCUUGA
276	UCCUGUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
301	CCAGGGG CUGAUGAGGCCGAAAGGCCGAA AUUCGAA
303	GACCAGG CUGAUGAGGCCGAAAGGCCGAA AGAUUCG
310	CCUUGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
323	UCAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
326	GGCCGGU CUGAUGAGGCCGAAAGGCCGAA AGGUGGA
335	UGUGGAAU CUGAUGAGGCCGAAAGGCCGAA AGGCCGG
349	UCCCCAC CUGAUGAGGCCGAAAGGCCGAA AGUUCAU
352	GCUGUUC CUGAUGAGGCCGAAAGGCCGAA AUGAUCU
375	CUCAUAG CUGAUGAGGCCGAAAGGCCGAA AGCCAUC
376	CUCCGGA CUGAUGAGGCCGAAAGGCCGAA AGACCRU
378	AGCCCUA CUGAUGAGGCCGAAAGGCCGAA AGUAGCC
391	CUGGGCA CUGAUGAGGCCGAAAGGCCGAA AGGUCAG
409	AGCUUAUG CUGAUGAGGCCGAAAGGCCGAA AUACUGC
416	CUAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUGGG
417	GUUCUGG CUGAUGAGGCCGAAAGGCCGAA AGCUAUG
418	GGUUCUG CUGAUGAGGCCGAAAGGCCGAA AAGCUAU
433	CACACUG CUGAUGAGGCCGAAAGGCCGAA AUCCCCA
467	CGAACAG CUGAUGAGGCCGAAAGGCCGAA AGCCUGG
469	GCUGGGU CUGAUGAGGCCGAAAGGCCGAA AUGGCUU
473	CUGAUCU CUGAUGAGGCCGAAAGGCCGAA ACUAAA
481	UGGUCUG CUGAUGAGGCCGAAAGGCCGAA AUUCGCU

501 AACGUGA CUGAUGAGGCCGAAAGGCCGAA AGGGGU
 502 GAACGUG CUGAUGAGGCCGAAAGGCCGAA AAGGGGU
 508 CUAUAGG CUGAUGAGGCCGAAAGGCCGAA ACGUGAA
 509 UCTAUAG CUGAUGAGGCCGAAAGGCCGAA AACGUGA
 512 UCCUCUA CUGAUGAGGCCGAAAGGCCGAA AGGAACG
 514 GCUCCUC CUGAUGAGGCCGAAAGGCCGAA AUAGGAA
 534 CAAGUCA CUGAUGAGGCCGAAAGGCCGAA AGUCCCC
 556 GGAAGCA CUGAUGAGGCCGAAAGGCCGAA AGGCGCA
 561 CACCUGG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
 562 UCACCUG CUGAUGAGGCCGAAAGGCCGAA AAGCAGA
 585 GCUGGCU CUGAUGAGGCCGAAAGGCCGAA AUGGUU
 598 UCAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
 613 GUGAGAG CUGAUGAGGCCGAAAGGCCGAA ACAGGGG
 616 GAUGUGA CUGAUGAGGCCGAAAGGCCGAA AGGACAG
 617 GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGGAC
 620 CAUGGCU CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
 623 GAGAUGG CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
 628 UAUCAAA CUGAUGAGGCCGAAAGGCCGAA AUCCGAU
 630 GUUAUCA CUGAUGAGGCCGAAAGGCCGAA AAAUCGG
 631 GGUUUAUC CUGAUGAGGCCGAAAGGCCGAA AAAAUCG
 638 GGAACAC CUGAUGAGGCCGAAAGGCCGAA AUGGCCA
 661 AGAUUU CUGAUGAGGCCGAAAGGCCGAA AGCUUCCG
 667 CUCCGGCA CUGAUGAGGCCGAAAGGCCGAA AUCUUGA
 687 GCUCOCA CUGAUGAGGCCGAAAGGCCGAA AGUUCCG
 700 CCCACCC CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
 715 GCAAGAA CUGAUGAGGCCGAAAGGCCGAA AUCUCAU
 717 CAGCAAG CUGAUGAGGCCGAAAGGCCGAA AGAUCUC
 718 ACAGCAA CUGAUGAGGCCGAAAGGCCGAA AAGAUCU
 721 CGCAADG CUGAUGAGGCCGAAAGGCCGAA AGGAGAA
 751 ACACCUC CUGAUGAGGCCGAAAGGCCGAA AUGUCUU
 759 CGUGAAA CUGAUGAGGCCGAAAGGCCGAA ACACCUC
 761 CCGUGUA CUGAUGAGGCCGAAAGGCCGAA AUACACC
 762 UCCCUGG CUGAUGAGGCCGAAAGGCCGAA AAUACAC
 763 GUCCCGU CUGAUGAGGCCGAAAGGCCGAA AAAUACA
 792 AGAAAAG CUGAUGAGGCCGAAAGGCCGAA AGCCUCG
 795 UUGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGAGCC
 796 CUUGAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAGC
 797 GCUUGAG CUGAUGAGGCCGAAAGGCCGAA AAAGGAG
 798 AGCUUGA CUGAUGAGGCCGAAAGGCCGAA AAAAGGA
 829 GGAACAC CUGAUGAGGCCGAAAGGCCGAA AUGGCCA
 834 AGUCCGG CUGAUGAGGCCGAAAGGCCGAA ACACAAU
 835 GAGUCCG CUGAUGAGGCCGAAAGGCCGAA AACACAA
 845 GCGUACG CUGAUGAGGCCGAAAGGCCGAA AGGAGUC
 849 GUCCGGG CUGAUGAGGCCGAAAGGCCGAA ACGGAGG
 872 CGAACAG CUGAUGAGGCCGAAAGGCCGAA AGCCUGG
 883 GCAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUUGAA
 885 CUGCAUG CUGAUGAGGCCGAAAGGCCGAA AGACUUG
 905 CGAUCAG CUGAUGAGGCCGAAAGGCCGAA AGGCCGC
 906 GCGAUCA CUGAUGAGGCCGAAAGGCCGAA AAGGCCG

919	GCUCACU CUGAUGAGGCGAAAGGCCGAA AGCUCGC
936	GUACUGG CUGAUGAGGCGAAAGGCCGAA ACUCCAU
937	AQUACUG CUGAUGAGGCGAAAGGCCGAA AACUCCA
942	UGGCAAG CUGAUGAGGCGAAAGGCCGAA ACUGGAA
953	UCAUGUG CUGAUGAGGCGAAAGGCCGAA AUGAGGC
962	CGGUGGC CUGAUGAGGCGAAAGGCCGAA AUCAUCU
965	GUCUGGC CUGAUGAGGCGAAAGGCCGAA AGUACUG
973	UCUCUUC CUGAUGAGGCGAAAGGCCGAA AUCCGGU
986	ACUCUUG CUGAUGAGGCGAAAGGCCGAA AGGUCUC
996	GGJCUCA CUGAUGAGGCGAAAGGCCGAA AGGUCCU
1005	ACUCUUG CUGAUGAGGCGAAAGGCCGAA AGGUUC
1006	UACUCUU CUGAUGAGGCGAAAGGCCGAA AAGGUCCU
1015	UCUUCAU CUGAUGAGGCGAAAGGCCGAA AUACUCU
1028	UUGAAAG CUGAUGAGGCGAAAGGCCGAA ACUCUUC
1031	CCAUUGA CUGAUGAGGCGAAAGGCCGAA AGGACUC
1032	UCCADUG CUGAUGAGGCGAAAGGCCGAA AAGGACU
1033	GUCCAUU CUGAUGAGGCGAAAGGCCGAA AAAGGAC
1058	CGGGUUG CUGAUGAGGCGAAAGGCCGAA AGGCCGG
1064	UUGGAUC CUGAUGAGGCGAAAGGCCGAA AGGUGUA
1072	GCACAGC CUGAUGAGGCGAAAGGCCGAA AUACGCC
1082	UUUCGGG CUGAUGAGGCGAAAGGCCGAA AGGCACA
1083	ACUUUCG CUGAUGAGGCGAAAGGCCGAA AAGGCUU
1092	AGAAGUU CUGAUGAGGCGAAAGGCCGAA AGUUUOG
1097	GGGACAG CUGAUGAGGCGAAAGGCCGAA AGUUGAG
1098	GGGGACA CUGAUGAGGCGAAAGGCCGAA AAGUUGA
1102	GCUUGGG CUGAUGAGGCGAAAGGCCGAA ACAGAAG
1125	GAAGGUG CUGAUGAGGCGAAAGGCCGAA AGGGCUG
1127	GUAAGGC CUGAUGAGGCGAAAGGCCGAA AUADGCC
1131	UGGUGCU CUGAUGAGGCGAAAGGCCGAA AGGGAG
1132	AUGCUGG CUGAUGAGGCGAAAGGCCGAA AAGGUGU
1133	GAAGCUG CUGAUGAGGCGAAAGGCCGAA AGAUUGA
1137	GCGCGCU CUGAUGAGGCGAAAGGCCGAA AAGUAAA
1140	GCUGAGG CUGAUGAGGCGAAAGGCCGAA AUGCUGG
1153	CAAAGUU CUGAUGAGGCGAAAGGCCGAA AUGGUGC
1158	CUCAUCA CUGAUGAGGCGAAAGGCCGAA AGUUGAU
1167	GGGGGAA CUGAUGAGGCGAAAGGCCGAA ACUCAUC
1168	UGGGGGG CUGAUGAGGCGAAAGGCCGAA AACUCAU
1169	AUGGGGG CUGAUGAGGCGAAAGGCCGAA AAACUCA
1182	UGAUGGU CUGAUGAGGCGAAAGGCCGAA ACAGCAU
1183	CUGAUGG CUGAUGAGGCGAAAGGCCGAA AACAGCA
1184	UCAGGAG CUGAUGAGGCGAAAGGCCGAA AGGGGCC
1187	GGCUGAG CUGAUGAGGCGAAAGGCCGAA AAGGGAC
1188	CUGCCCC CUGAUGAGGCGAAAGGCCGAA AUGCUGA
1198	UCAGACU CUGAUGAGGCGAAAGGCCGAA AACUCCC
1209	GAAGGUG CUGAUGAGGCGAAAGGCCGAA AGGGCUG
1215	CGGUGCU CUGAUGAGGCGAAAGGCCGAA AGGCCAG
1229	GCUGAGG CUGAUGAGGCGAAAGGCCGAA AGGGACC
1237	GGGGCAG CUGAUGAGGCGAAAGGCCGAA AGCUGGG
1250	GAGCCUG CUGAUGAGGCGAAAGGCCGAA AGGCUGG

1268	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
1279	AGGAAGG CUGAUGAGGCCGAAAGGCCGAA ACCAUGG
1281	CGCAGCU CUGAUGAGGCCGAAAGGCCGAA AGCCCAC
1286	UGGGGGA CUGAUGAGGCCGAAAGGCCGAA AACUCAU
1309	AGACTUCG CUGAUGAGGCCGAAAGGCCGAA ACAGGAG
1315	GGGUUAG CUGAUGAGGCCGAAAGGCCGAA ACUGGGG
1318	CCGGGGU CUGAUGAGGCCGAAAGGCCGAA AGAACUG
1331	GACUGGG CUGAUGAGGCCGAAAGGCCGAA AGGACCC
1334	UCAGCUU CUGAUGAGGCCGAAAGGCCGAA AGAAAAG
1389	GGCUUCC CUGAUGAGGCCGAAAGGCCGAA ACAGCGU
1413	AGCAUCA CUGAUGAGGCCGAAAGGCCGAA ACUGCAG
1414	CAGCAUC CUGAUGAGGCCGAAAGGCCGAA AACUGCA
1437	GCCAAGC CUGAUGAGGCCGAAAGGCCGAA AGGCCCC
1441	UGUUGCC CUGAUGAGGCCGAAAGGCCGAA AGCAAGG
1467	GUCUGUG CUGAUGAGGCCGAAAGGCCGAA ACACUCC
1468	GGUCUGU CUGAUGAGGCCGAAAGGCCGAA AACACUC
1482	GUCCACA CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
1486	AGUUCCC CUGAUGAGGCCGAAAGGCCGAA ACGAACG
1494	AAACUCU CUGAUGAGGCCGAAAGGCCGAA AGUUGUC
1500	CUGCUGA CUGAUGAGGCCGAAAGGCCGAA ACUCUGA
1501	GCUGCUG CUGAUGAGGCCGAAAGGCCGAA AACUCUG
1502	AGCUGCU CUGAUGAGGCCGAAAGGCCGAA AAACUCU
1525	ACACAGG CUGAUGAGGCCGAAAGGCCGAA AUGCACC
1566	UUCAGGG CUGAUGAGGCCGAAAGGCCGAA ACUCCAU
1577	CGAGUUA CUGAUGAGGCCGAAAGGCCGAA AGCUUCA
1579	GGCGAGU CUGAUGAGGCCGAAAGGCCGAA AUAGCUU
1583	ACCAGGC CUGAUGAGGCCGAAAGGCCGAA AGUUAUA
1588	CCCUCUC CUGAUGAGGCCGAAAGGCCGAA AGGAGAG
1622	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
1628	CCUACCG CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
1648	CAUUGGG CUGAUGAGGCCGAAAGGCCGAA AGCCCOG
1660	CUGGGCA CUGAUGAGGCCGAAAGGCCGAA AGGUCAG
1663	CACCTUGG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
1664	UCACCUUG CUGAUGAGGCCGAAAGGCCGAA AAGCAGA
1665	ACCUCCG CUGAUGAGGCCGAAAGGCCGAA AAGCGAG
1680	GGAGGAG CUGAUGAGGCCGAAAGGCCGAA AGUCUUC
1681	UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AAGUCUU
1683	AAUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGUC
1686	CGCAAUG CUGAUGAGGCCGAAAGGCCGAA AGGAGAA
1690	UGUOCGC CUGAUGAGGCCGAAAGGCCGAA AUGGAGG
1704	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGUCCAU
1705	GAGCAGA CUGAUGAGGCCGAAAGGCCGAA AAGUCCA
1707	AAGAGCA CUGAUGAGGCCGAAAGGCCGAA AGAAGUC
1721	CUGAUCU CUGAUGAGGCCGAAAGGCCGAA ACUAAA
1726	AGGAGCU CUGAUGAGGCCGAAAGGCCGAA AUCUGAC
1731	ACCUUAG CUGAUGAGGCCGAAAGGCCGAA AGCUGAU
1734	AGCACCU CUGAUGAGGCCGAAAGGCCGAA AGGAGCU
1754	CUCUUGG CUGAUGAGGCCGAAAGGCCGAA AGCACUG

Table 20
 Human *rel A* HH Ribozyme Sequences
 nt. Position HH Ribozyme Sequences

19	UACAGAC CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
22	CACUACA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC
26	CGUGGCAC CUGAUGAGGCCGAAAGGCCGAA ACAGACG
93	GAGGGGG CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
94	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA AACAGUU
100	GGAGAU CUGAUGAGGCCGAAAGGCCGAA AGGGGGA
103	CCCCGAA CUGAUGAGGCCGAAAGGCCGAA AUGAGGG
105	UGCCGG CUGAUGAGGCCGAAAGGCCGAA AGAUGAG
106	CUGCCGG CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
129	GGGGCCA CUGAUGAGGCCGAAAGGCCGAA AGGCCUG
138	CUCCACA CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
148	GCUAAU CUGAUGAGGCCGAAAGGCCGAA AUUCUCA
151	GCUGCUC CUGAUGAGGCCGAAAGGCCGAA AUGAUCU
180	GUAGCGG CUGAUGAGGCCGAAAGGCCGAA AGOGCAU
181	UGUAGCG CUGAUGAGGCCGAAAGGCCGAA AAGOGCA
186	GCACUUG CUGAUGAGGCCGAAAGGCCGAA AGCGGAA
204	GCCCCGG CUGAUGAGGCCGAAAGGCCGAA AGCGCCC
217	CGCCUGG CUGAUGAGGCCGAAAGGCCGAA AUGCUGC
239	UUGGUGG CUGAUGAGGCCGAAAGGCCGAA AUUCUGU
262	UGAUCUU CUGAUGAGGCCGAAAGGCCGAA AUUGGUGG
268	AGCCAUU CUGAUGAGGCCGAAAGGCCGAA AUUCUGA
276	UCCUGUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUU
301	CCAGGGG CUGAUGAGGCCGAAAGGCCGAA AUUGGCCA
303	GACCAGG CUGAUGAGGCCGAAAGGCCGAA AGAUGCG
310	CCUUGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
323	GGGUGAG CUGAUGAGGCCGAAAGGCCGAA AGGGUCC
326	GGCCGGU CUGAUGAGGCCGAAAGGCCGAA AGGAGGG
335	UGGGGGU CUGAUGAGGCCGAAAGGCCGAA AGGCCGG
349	UUCCUAC CUGAUGAGGCCGAAAGGCCGAA ACCUCGU
352	CCUUUUC CUGAUGAGGCCGAAAGGCCGAA ACAAGCU
375	CUCAUAG CUGAUGAGGCCGAAAGGCCGAA AGCCAU
376	CCUCAUU CUGAUGAGGCCGAAAGGCCGAA AAGCCAU
378	AGCCUCA CUGAUGAGGCCGAAAGGCCGAA AGAACCC
391	CCGGGCA CUGAUGAGGCCGAAAGGCCGAA AGCUCAG
409	AACUGUG CUGAUGAGGCCGAAAGGCCGAA AUGCAGC
416	UUCUGGA CUGAUGAGGCCGAAAGGCCGAA ACUGUGG
417	GUUCUGG CUGAUGAGGCCGAAAGGCCGAA AACUGUG
418	GGUUCUG CUGAUGAGGCCGAAAGGCCGAA AAACUGU
433	CACACUG CUGAUGAGGCCGAAAGGCCGAA AUUCCCA
467	UGACUGA CUGAUGAGGCCGAAAGGCCGAA AGCCUGC
469	GCUGACU CUGAUGAGGCCGAAAGGCCGAA AUAGCCU
473	AUGOGCU CUGAUGAGGCCGAAAGGCCGAA ACUGAU
481	UGGUCUG CUGAUGAGGCCGAAAGGCCGAA AUGOGCU
501	AACUUGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUU

502 GAACUUG CUGAUGAGGCCGAAAGGCCGAA AAGGGGU
 508 CUADAGG CUGAUGAGGCCGAAAGGCCGAA ACUUGGA
 509 UCUAUAG CUGAUGAGGCCGAAAGGCCGAA AACUUGG
 512 UCUUCUA CUGAUGAGGCCGAAAGGCCGAA AGGAACU
 514 GCUCUUC CUGAUGAGGCCGAAAGGCCGAA AUAGGAA
 534 CAGGUCC CUGAUGAGGCCGAAAGGCCGAA AGUCCCC
 556 GGAAGCA CUGAUGAGGCCGAAAGGCCGAA AGCGCGA
 561 CACCUUG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
 562 UCACCUG CUGAUGAGGCCGAAAGGCCGAA AAGCAGA
 585 CCUGCCU CUGAUGAGGCCGAAAGGCCGAA AUAGGUC
 598 GCAGGCG CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
 613 GAGGAAG CUGAUGAGGCCGAAAGGCCGAA ACAGGCG
 616 GAUGAGG CUGAUGAGGCCGAAAGGCCGAA AGGACAG
 617 GGAUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGACA
 620 AUGGGAU CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
 623 AAGAUGG CUGAUGAGGCCGAAAGGCCGAA AUGAGGA
 628 UGUCAAA CUGAUGAGGCCGAAAGGCCGAA AUAGGAG
 630 AUUGUCA CUGAUGAGGCCGAAAGGCCGAA AGAUAGG
 631 GAUUGUC CUGAUGAGGCCGAAAGGCCGAA AAGAUGG
 638 GGGGCAC CUGAUGAGGCCGAAAGGCCGAA AUUGUCA
 661 AGAUCUU CUGAUGAGGCCGAAAGGCCGAA AGCUCCG
 667 CUCGGCA CUGAUGAGGCCGAAAGGCCGAA AUCUUGA
 687 GCUGCCA CUGAUGAGGCCGAAAGGCCGAA AGUUUCG
 700 CCCACC CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
 715 GUAGGAA CUGAUGAGGCCGAAAGGCCGAA AUCUCAU
 717 CAGUAGG CUGAUGAGGCCGAAAGGCCGAA AGAUCUC
 718 ACAGUAG CUGAUGAGGCCGAAAGGCCGAA AAGAUCU
 721 CACACAG CUGAUGAGGCCGAAAGGCCGAA AGGAAGA
 751 ACACCCUC CUGAUGAGGCCGAAAGGCCGAA AUGUCCU
 759 CGUGAAA CUGAUGAGGCCGAAAGGCCGAA ACACCCUC
 761 CCCGUGA CUGAUGAGGCCGAAAGGCCGAA AUACACC
 762 UCCCUGG CUGAUGAGGCCGAAAGGCCGAA AAUACAC
 763 GUCCCGU CUGAUGAGGCCGAAAGGCCGAA AAAUACA
 792 CGAAAAG CUGAUGAGGCCGAAAGGCCGAA AGCCUOG
 795 UUGCGAA CUGAUGAGGCCGAAAGGCCGAA AGGAGCC
 796 CUUGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGAGC
 797 GCUUGCG CUGAUGAGGCCGAAAGGCCGAA AAAGGAG
 798 AGCUUGC CUGAUGAGGCCGAAAGGCCGAA AAAAGGA
 829 GGAACAC CUGAUGAGGCCGAAAGGCCGAA AUGGCCA
 834 GGUCCCG CUGAUGAGGCCGAAAGGCCGAA ACACAAU
 835 GGGUCCG CUGAUGAGGCCGAAAGGCCGAA AACACAA
 845 GCGUAGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUC
 849 GUCUGCG CUGAUGAGGCCGAAAGGCCGAA AGGGAGG
 872 CGCACAG CUGAUGAGGCCGAAAGGCCGAA AGCCUGC
 883 GCAUGGA CUGAUGAGGCCGAAAGGCCGAA ACAOGCA
 885 CUGCAUG CUGAUGAGGCCGAAAGGCCGAA AGACAOG
 905 CGGUCCG CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
 906 CGGUUCG CUGAUGAGGCCGAAAGGCCGAA AAGGCOG
 919 GCUCACU CUGAUGAGGCCGAAAGGCCGAA AGCUCCC

936 GUACUGG CUGAUGAGGCCGAAAGGCCGAA AUUCCAU
 937 GGUACUG CUGAUGAGGCCGAAAGGCCGAA AAUUCCA
 942 UGGCAGG CUGAUGAGGCCGAAAGGCCGAA ACUGGAA
 953 UCGUCUG CUGAUGAGGCCGAAAGGCCGAA AUCUGGC
 962 CGGUGAC CUGAUGAGGCCGAAAGGCCGAA AUCGUCU
 965 AUCCGGU CUGAUGAGGCCGAAAGGCCGAA ACGAUCG
 973 UCUCUC CUGAUGAGGCCGAAAGGCCGAA AUCCGGU
 986 GUCCUUU CUGAUGAGGCCGAAAGGCCGAA ACGUUUC
 996 GGUCUCA CUGAUGAGGCCGAAAGGCCGAA ADGUCCU
 1005 GCUCUUG CUGAUGAGGCCGAAAGGCCGAA AGGUCCG
 1006 UGCUCUU CUGAUGAGGCCGAAAGGCCGAA AAGGUCCJ
 1015 UCUUCAU CUGAUGAGGCCGAAAGGCCGAA AUGCUCJ
 1028 CUGAAAG CUGAUGAGGCCGAAAGGCCGAA ACUCUJC
 1031 CCGCUGA CUGAUGAGGCCGAAAGGCCGAA AGGACUC
 1032 UCCCGUG CUGAUGAGGCCGAAAGGCCGAA AAGGACU
 1033 GUCCGCU CUGAUGAGGCCGAAAGGCCGAA AAAGGAC
 1058 CGAGGUG CUGAUGAGGCCGAAAGGCCGAA AGGCCGG
 1064 AUGCGUC CUGAUGAGGCCGAAAGGCCGAA AGGUGGA
 1072 GCACAGC CUGAUGAGGCCGAAAGGCCGAA AUGCGUC
 1082 CUGCGGG CUGAUGAGGCCGAAAGGCCGAA AGGCACA
 1083 GCDUGGG CUGAUGAGGCCGAAAGGCCGAA AAGGCAC
 1092 AGAACGU CUGAUGAGGCCGAAAGGCCGAA AGCUGCG
 1097 GGGACAG CUGAUGAGGCCGAAAGGCCGAA AGCUGAG
 1098 GGGGACA CUGAUGAGGCCGAAAGGCCGAA AAGCUGA
 1102 GCUUUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
 1125 AAAGGGA CUGAUGAGGCCGAAAGGCCGAA AGGGCUG
 1127 GUAAAAG CUGAUGAGGCCGAAAGGCCGAA AUAGGGC
 1131 UGACGUA CUGAUGAGGCCGAAAGGCCGAA AGGGAU
 1132 AUGACGU CUGAUGAGGCCGAAAGGCCGAA AAGGGAU
 1133 GAUGACG CUGAUGAGGCCGAAAGGCCGAA AAAGGGA
 1137 CAGGGAU CUGAUGAGGCCGAAAGGCCGAA ACGUAAA
 1140 GCUCAGG CUGAUGAGGCCGAAAGGCCGAA AUGACGU
 1153 CAUAGUU CUGAUGAGGCCGAAAGGCCGAA AUGGUGC
 1158 CUCAUCA CUGAUGAGGCCGAAAGGCCGAA AGUUGAU
 1167 GGUGGG A CUGAUGAGGCCGAAAGGCCGAA ACUCAUC
 1168 UGGUGGG CUGAUGAGGCCGAAAGGCCGAA AACUCAU
 1169 AUGGUGG CUGAUGAGGCCGAAAGGCCGAA AAACUCA
 1182 AGAAGGA CUGAUGAGGCCGAAAGGCCGAA ACACCAU
 1183 CAGAAGG CUGAUGAGGCCGAAAGGCCGAA AACACCA
 1184 CCAGAAG CUGAUGAGGCCGAAAGGCCGAA AACACCC
 1187 UGCCAG CUGAUGAGGCCGAAAGGCCGAA AGGAAAC
 1188 CUGOCGA CUGAUGAGGCCGAAAGGCCGAA AAGGAAA
 1198 CCUGGCU CUGAUGAGGCCGAAAGGCCGAA AUCUGCC
 1209 CAAGGCC CUGAUGAGGCCGAAAGGCCGAA AGGOCUG
 1215 CGGGGCC CUGAUGAGGCCGAAAGGCCGAA AGGCCGA
 1229 ACUUGGG CUGAUGAGGCCGAAAGGCCGAA AGGGGCC
 1237 GGGCAG CUGAUGAGGCCGAAAGGCCGAA ACUUGGG
 1250 GGGCUG CUGAUGAGGCCGAAAGGCCGAA AGCCUGG
 1268 AUGGCUG CUGAUGAGGCCGAAAGGCCGAA AGCAGGG

1279	GAGCUGA CUGAUGAGGCCGAAAGGCCGAA ACCAUGG
1281	CAGAGCU CUGAUGAGGCCGAAAGGCCGAA AUACCAU
1286	UGGGCCA CUGAUGAGGCCGAAAGGCCGAA AGCUGAU
1309	GGACUGG CUGAUGAGGCCGAAAGGCCGAA ACAGGGG
1315	GGGCUAG CUGAUGAGGCCGAAAGGCCGAA ACUGGGA
1318	CUGGGGC CUGAUGAGGCCGAAAGGCCGAA AGGACUG
1331	GCCUGAG CUGAUGAGGCCGAAAGGCCGAA AGGCCU
1334	ACAGCCU CUGAUGAGGCCGAAAGGCCGAA AGGAGGG
1389	GGCCUCU CUGAUGAGGCCGAAAGGCCGAA ACAGCGU
1413	AUCAUCA CUGAUGAGGCCGAAAGGCCGAA ACUGCAG
1414	CAUCACU CUGAUGAGGCCGAAAGGCCGAA AACUGCA
1437	GCCAAGC CUGAUGAGGCCGAAAGGCCGAA AGGCCCC
1441	UGUUGCC CUGAUGAGGCCGAAAGGCCGAA AGCAAGG
1467	GUCUGUG CUGAUGAGGCCGAAAGGCCGAA ACACAGC
1468	GGUCUGU CUGAUGAGGCCGAAAGGCCGAA AACACAG
1482	GUCGACG CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
1486	AGUUGUC CUGAUGAGGCCGAAAGGCCGAA ACGGAUG
1494	AAACUUG CUGAUGAGGCCGAAAGGCCGAA AGUUGUC
1500	CUGCUGA CUGAUGAGGCCGAAAGGCCGAA ACUOGGA
1501	GCUGCUG CUGAUGAGGCCGAAAGGCCGAA AACUCGG
1502	AGCUGCU CUGAUGAGGCCGAAAGGCCGAA AAACUCG
1525	CCACAGG CUGAUGAGGCCGAAAGGCCGAA AUGCCU
1566	CUCAGGG CUGAUGAGGCCGAAAGGCCGAA ACUCCAU
1577	CGAGUUA CUGAUGAGGCCGAAAGGCCGAA AGCCUCA
1579	GGCGAGU CUGAUGAGGCCGAAAGGCCGAA AUAGCCU
1583	ACUAGGC CUGAUGAGGCCGAAAGGCCGAA AGUUAUU
1586	CUGUCAC CUGAUGAGGCCGAAAGGCCGAA AGGCGAG
1622	GGAGCAG CUGAUGAGGCCGAAAGGCCGAA AGCUGGG
1628	CCCAGUG CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
1648	CAUUGGG CUGAUGAGGCCGAAAGGCCGAA AGCCCCG
1660	CUGAAAG CUGAUGAGGCCGAAAGGCCGAA AGGCCAU
1663	CUCCUGA CUGAUGAGGCCGAAAGGCCGAA AGGAGCC
1664	UCUCCUG CUGAUGAGGCCGAAAGGCCGAA AAGGAGG
1665	AUCUCU CUGAUGAGGCCGAAAGGCCGAA AAAGGAG
1680	GGAGGAG CUGAUGAGGCCGAAAGGCCGAA AGUCUUC
1681	UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AAGUCUU
1683	AAUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAACUC
1686	CGCAAUG CUGAUGAGGCCGAAAGGCCGAA AGGAGAA
1690	UGUCCGC CUGAUGAGGCCGAAAGGCCGAA Auggagg
1704	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AGUCCAU
1705	GGGCUUA CUGAUGAGGCCGAAAGGCCGAA AAGUCCA
1707	CAGGGCU CUGAUGAGGCCGAAAGGCCGAA AGAACUC
1721	CUGAUCU CUGAUGAGGCCGAAAGGCCGAA ACUCAGC
1726	AGGAGCU CUGAUGAGGCCGAAAGGCCGAA AUUGAC
1731	CCCUCUAG CUGAUGAGGCCGAAAGGCCGAA AGCUGAU
1734	ACCCCCU CUGAUGAGGCCGAAAGGCCGAA AGGAGCU
1754	CUCUGGG CUGAUGAGGCCGAAAGGCCGAA AGGGCAG

Table 21
Human *relA* Hairpin Ribozyme Target Sequences
nt. Position

	Substrate
90	UGGGGG AGAA GUUC ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
156	CCUGCUG AGAA GCUC ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
362	GCCAUCCC AGAA GUCC ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
413	GUUCUGGA AGAA GUGG ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
606	GAAGGACA AGAA GCAG ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
652	UGAGCUC AGAA GUGU ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
695	CCCACCGA AGAA GCUG ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
853	AGGTUGGG AGAA GCGU ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
900	GGUCGGAA AGAA GCGG ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
955	UGACGAUC AGAA GUAU ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
1037	GUCCGUCC AGAA GCUG ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
1045	GGCCGGGG AGAA GUGG ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
1410	CAUCAUCA AGAA GCAG ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
1453	ACAGGUGG AGAA GUGC ACCAGAGAACACCAUUCGUUACAUUACCUUGUA
1471	GAUCCACG AGAA GUGA ACCAGAGAACACCAUUCGUUACAUUACCUUGUA

Table 22
Mouse *relA* Hairpin Ribozyme Target Sequences
nt. Position

		Substrate
137	GUGUCUUC AGAA GUUC ACCAGAGAAACACACCGUUGGGUACAUUCCUGUA	GAACA GCC GAAGCAAC
273	GAGAUUCG AGAA GUUC ACCAGAGAAACACACGUUGGGUACAUUCCUGUA	GAACA GGU CGAAUCUC
343	GCCAUCCC AGAA GUCC ACCAGAGAAACACACGUUGGGUACAUUCCUGUA	GSGACU CCC GGGAUGGC
366	GGCCGAG AGAA GCCU ACCAGAGAAACACACGUUGGGUACAUUCCUGUA	AGGCCU GAC CUCUGGCC
633	UGGACCTC AGAA GUGU ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	AACAU GCC GAGGUCAA
676	CCCACCCA AGAA GCUC ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	GAGCU GCC UCGGGGGG
834	AGGCUCCG AGAA GGCU ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	ACTGC GAC CCCAGCCU
881	GAUCRCAA AGAA GCGG ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	CCTCC GCC UCTUGAUC
1100	AGGTGTAG AGAA GCGG ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	CCGCA GCC CUACACCU
1205	GGGCAAGG AGAA GUCC ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	GCACC GUC CUCUGGCC
1361	GGGCTUCC AGAA GGCU ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	ACGCCU GUC GGAAGCCC
1385	CAGCAUCA AGAA GCGG ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	CUGCA GTU UGAUGCUA
1431	ACUCCUGG AGAA GUCC ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	GCACA GAC CCAGGAGU
1449	GAUGCAG AGAA GUGA ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	UCACA GAC CUGGCAUC
1802	AAGUCCCC AGAA GGCG ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	CAGCU GCC CCCAGCUU
2009	UGGCUCCA AGAA GUCC ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	GGACA GAC UGGAGCCA
2124	UGGUGUCG AGAA GCAC ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	GUCCU GCC CGACACCA
2233	AUUCUGAA AGAA GCCA ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	UGGCC GCC UUCAGAAU
2354	UCAGUAAA AGAA GUCU ACCAGAGAAACACCGUUGGGUACAUUCCUGUA	AGACA GCC UUUACUGA

Table 23: Human TNF- α HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
28	GCCAGGU U CUCUUCC	321	GUCAGAU C AUCCUCU
29	GCAGGUU C UCUCUCC	324	AGAUCAU C UUCUCGA
31	AGGUUCU C UUCCUCU	326	AUCAUCU U CUCGAAC
33	GUUCUCU U CCUCUCA	327	UCAUCU C UCGAAC
34	UUCGCUU C CUCUCAC	329	AUCUUUC U GAAACCC
37	UCUUCU C UCACAUU	352	AGCCUGU A GCCC AUG
39	UUCUCU C ACAUACU	361	CCAUJGU U GUAGCAA
44	CUCACAU A CUGACCC	364	ADGUUGU A GCAAACC
58	CAOGGCU C CACCCUC	374	AAACCCU C AACUGA
65	CCACCCU C UCUCCCC	391	GGCAGGU C CAGGGC
67	ACCCUCU C UCCCCUG	421	ADGCCCU C CUGGCCA
69	CCUCUCU C CCCUGGA	449	GAGAGAU A ACCAGCU
106	GCAUGAU C CGGGACG	468	GUGCCAU C AGAGGGC
136	AGGOGCU C CCCAAGA	480	GGCCUGU A CCUCAU
165	CAGGGCU C CAGGCCG	484	UGUACCU C AUCUACU
177	CGGUGGU U GUUCCUC	487	ACCUCAU C UACUCCC
180	UGCUGGU U CCUCAGC	489	CUCAUCAU A CUCCAG
181	GCUUGUU C CUCAGCC	492	AUCUACU C CCAGGUC
184	UGUUCUU C AGOCUCU	499	CCCAGGU C CUCUUA
190	UCAGGCCU C UUCUCUU	502	AGGUCCU C UUCAAGG
192	AGCCUCU U CUCCUUC	504	GUCCUCU U CAAGGGC
193	GCCUCUU C UCCUUDCC	505	UCCUCUU C AAGGGCC
195	CUCUUCU C CUUOCUG	525	UGCCCCU C CACCCAU
198	UUCUCUU U CCTGAUC	538	AUGUGGU C CUCACCC
199	UCUCUUU C CUGACCG	541	UGCUCUU C ACCCACA
205	UCCUGAU C GUGGCAG	553	ACACCAU C AGCGCA
226	CCACGGU C UUCUGCC	562	GCGGCAU C GCCGUC
228	ACGCUCU U CUGOCUG	568	UCGCGGU C UCCUACC
229	CCUCUUU C UGCGUGC	570	GCGGUU C CUACAG
243	CUGCACU U UGGAGUG	573	GUCUCCU A CCAGACC
244	UGCACUU U GGAGUGA	586	CCAAGGU C AACCUCC
253	GAGUGAU C GGCCCCC	592	UCAACCU C CUCUCUG
273	GAAGAGU C CCCAGG	595	ACCUCCU C UCUGCCA
286	GGGACCU C UCUCUAA	597	CUCCUCU C UGCCAUC
288	GACCUU C UCUAUUC	604	CUGCCAU C AAGAGCC
290	CCUCUCU C UAAUCAG	657	CCCUGGU A UGAGGCC
292	UCUCUCU A AUCAGCC	667	AGCCCCAU C UAUCUGG
295	CUCUAAU C AGCCUC	669	CCCAUCU A UCUGGGA
302	CAOGCCCU C UGGCCCA		

671	CAUCUAU C UGGGAGG	960	UGGGAUU C AGGAAUG
682	GAGGGGU C UUCCAGC	1001	AACCACU A AGAAUUC
684	GGGGUCU U CCAGCUG	1007	UAAAUAU U CAAACUG
685	GGGUUU C CAGCUGG	1008	AAGAAUU C AAACUGG
709	ACCGACU C AGCGCUG	1021	GGGGCCU C CAGAACU
721	CUGAGAU C AAUOGGC	1029	CAGAACU C ACUGGGG
725	GAUCAAU C GGCCCCA	1040	GGGGCCU A CAGCUUU
735	CCCGACU A UCUCGAC	1046	UACAGCU U UGAUCCC
737	CGACUAU C UCGACUU	1047	ACAGCUU U GAUCCCU
739	ACUACUC C GACUUUUG	1051	CUUUGAU C CCUGACA
744	CUCGACU U UGCCGAG	1060	CUGACAU C UGGAAUC
745	UCGACUU U GCGAGU	1067	CUGGAAU C UGGAGAC
753	GCGGAGU C UGGGCAG	1085	GGAGOCU U UGGJUCU
763	GGCAGGU C UACUUUG	1086	GAGCCUU U GGUUCTUG
765	CAGGUCU A CUUUGGG	1090	CUUUGGU U CUGGCCA
768	GUCUACU U UGGGAUC	1091	UUUGGUU C UGGCCAG
769	UCUACUU U GGGAUCA	1113	CAGGACU U GAGAAGA
775	UUGGGAU C AUUGCCC	1124	AAGACCU C ACCUAGA
778	GGAUCAU U GCGCUGU	1129	CUCACCU A GAAATUG
801	CGAACAU C CAACCUU	1135	UAGAAAU U GACACAA
808	CCAACCU U CCCAAC	1151	UGGACCU U AGGCCUU
809	CAACCUU C CCAAACG	1152	GGACCUU A GGCCUUC
820	AACGCCU C CCCUGCC	1158	UAGGCCU U CCUCUCU
833	CCCCAAU C CCUUUAU	1159	AGGOCUU C CUCUCUC
837	AAUCCCU U UAUUACC	1162	CCUUCGU C UCUCAG
838	AUCCCUU U AUUACCC	1164	UUCCUCU C UCCAGAU
839	UCCCUUU A UUACOCC	1166	CCUCUCU C CAGAUGU
841	CCUUUAU U ACCCCCCU	1174	CAGAUGU U UCCAGAC
842	CUUUAUJ A CCOCUC	1175	AGAUGUU U CCAGACU
849	ACCCCCU C CUUCAGA	1176	GAUGUUU C CAGACUU
852	CCUCUCU U CAGACAC	1183	CCAGACU U CCUUCAG
853	CCUCCUU C AGACRACC	1184	CAGACUU C CUUGAGA
863	ACACCCU C AACCUUC	1187	ACUUCCU U GAGACAC
869	UCAACCU C UUCUGGC	1208	CAGGCCU C CCCAUGG
871	AACCUCU U CUUGGCUC	1224	CCAGACU C CCUCUAU
872	ACCUCUU C UGGCUCA	1228	CCUCCCCU C UAUUUAU
878	UCUGGCCU C AAAAGAG	1230	UCCCUCU A UUUAGU
890	AGAGAAU U GGGGGCU	1232	CCUCUAU U UAUGUUU
898	GGGGGCU U AGGGUCG	1233	CUCUAUU U AUGUUG
899	GGGGCUU A GGGUCGG	1234	UCUAIUU A UGUUUGC
904	UUAGGGU C GGAACCC	1238	UUUAUGU U UGCACUU
917	CCAAGCU U AGAACUU	1239	UUUAUGU U GCACUUG
918	CAAGCUU A GAACUUU	1245	UUGCACU U GUGAUUA
924	UAGAACU U UAAGCAA	1251	UUGUGAU U AUUUAUU
925	AGAACUU U AAGCAAC	1252	UGUGAUU A UUUUAUA
926	GAACUUU A AGCAACA	1254	UGAUUAU U UAUUAUU
945	CACCAACU U CGAAACC	1255	GAUUAUJ U AUUAUU
946	ACCACUU C GAAACCU	1256	AUUAUJU A UUAUUA
959	CUGGGAU U CAGGAAU	1258	UAUUAUJ U AUUUAUU

1259	AUUAUUA UUUAUUU	1440	UGJUUUU U AAAAUAU
1261	UUAAUUA U UAUUUAU	1441	GCUUUUU A AAADAUU
1262	UAIUUAU U AUUUUAU	1446	UUAAAUA A UUACUG
1263	AUUAUUA A UUUAUUA	1448	AAAUAU U AUCUGAU
1265	UAUUAU U UAUUAU	1449	AAAUAU A UCTGAUU
1266	AUUAUUA U AUUAUUA	1451	AUAUUAU C UGAUUA
1267	UUUAUUA A UUADUUA	1456	AUCUGAU U AAGJUGU
1269	UAUUAU U AUUAUUA	1457	UCUGAU A AGJUGUC
1270	AUUAUUA A UUUAUUA	1461	AUUAAGU U GGCJAAA
1272	UUUAUUA U UAUUAU	1464	AAGUUGU C UAAACAA
1273	UAUUAU U AUUAUUA	1466	GUUGCU A AACAAUG
1274	AUUAUUA A UUUAUUA	1479	UGCUGAU U UGGUGAC
1276	UAUUAU U UAUUAU	1480	GCGGAUU U GGGGACC
1277	AUUAUUA U AUUAUCA	1494	CAACUGU C ACUCAU
1278	UUUAUUA A UUUACAG	1498	UGUCACU C AUCGUG
1280	UAUUAU U UACAGAU	1501	CACUCAU U GCGUGAG
1281	AUUAUUA U ACAGAUG	1512	GAGGCCU C UGCUCCC
1282	UUUAUUA A CAGADGA	1517	CUCUGCU C CCCAGGG
1294	UGAAGGU A UUUAUUA	1528	AGGGAGU U GUGUCUG
1296	AAUGUAU U UAUUUGG	1533	GUUGUGU C UGUAUAC
1297	AUGUAUU U ADUUGGG	1537	UGUCUGU A AUCCGCC
1298	UGUADUU A UUUGGGA	1540	CUGUAU C GGCCUAC
1300	UAUUAU U UGGGAGA	1546	UCGGCCU A CUAUCA
1301	AUUAUUA U GGGAGAC	1549	GCCUACU A UUCAGUG
1315	CCGGGGU A UCCUGGG	1551	CUACUAU U CAGUGGC
1317	GGGGUAU C CUGGGGG	1552	UACUAU C AGUGGCC
1334	CCAALGU A GGAGCUG	1566	GAGAAAU A AAGGUUG
1345	GCUGGCCU U GGCUCAG	1572	UAAAGGU U GCUUAGG
1350	CUUGGCU C AGACAU	1576	GGUUGCU U AGGAAAG
1359	GACAUGU U UUCCGUG	1577	GUUGCUU A GGAAAGA
1360	ACAUGUU U UCCGUGA		
1361	CAUGUUU U CCGUGAA		
1362	AUGUUUU C CGUGAAA		
1386	GAACAAU A GGCUGUU		
1393	AGGCUGU U CCCAUGU		
1394	GGCUGUU C CCAUGUA		
1401	CCCAUGU A GCCCCCU		
1414	CUGGCCU C UGUGGCCU		
1422	UGUGGCCU U CUUUGA		
1423	GUGCCUU C UUUUGAU		
1425	GCCUUCU U UUGAUUA		
1426	CCUUCUU U UGAUUAU		
1427	CUUCUUU U GAUUAUG		
1431	UUUUCAU U AUGUUUU		
1432	UUUGAUU A UGUUUUU		
1436	AUUAUGU U UUUAAAA		
1437	UUUAUGU U UUUAAAA		
1438	UAUGUUU U UUAAAUAU		

Table 24: Human TNF- α Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
28	GGAAGAG CUGAUGAGGCCGAAAGGCCGAA ACCUGCC
29	AGGAAGA CUGAUGAGGCCGAAAGGCCGAA AACCUUGC
31	AGAGGAA CUGAUGAGGCCGAAAGGCCGAA AGAACCU
33	UGAGAGG CUGAUGAGGCCGAAAGGCCGAA AGAGAAC
34	GUGAGAG CUGAUCAGGCCGAAAGGCCGAA AAGAGAA
37	UAUGUGA CUGAUGAGGCCGAAAGGCCGAA AGGAAGA
39	AGUAUGU CUGAUGAGGCCGAAAGGCCGAA AGAGGAA
44	GGGUUCAG CUGAUGAGGCCGAAAGGCCGAA AUGUGAG
58	GAGGGUG CUGAUGAGGCCGAAAGGCCGAA AGCCGUG
65	GGGGAGA CUGAUGAGGCCGAAAGGCCGAA AGGGUGG
67	CAGGGGA CUGAUGAGGCCGAAAGGCCGAA AGAGGGU
69	UCCAGGG CUGAUGAGGCCGAAAGGCCGAA AGAGAGG
106	CGUCCCG CUGAUGAGGCCGAAAGGCCGAA AUCAUGC
136	UCUUGGG CUGAUGAGGCCGAAAGGCCGAA AGOGCCU
165	CCGCCUG CUGAUGAGGCCGAAAGGCCGAA AGCCCUG
177	GAGGAAC CUGAUGAGGCCGAAAGGCCGAA AGCACCG
180	GCUGAGG CUGAUGAGGCCGAAAGGCCGAA ACAAGCA
181	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AACAAAGC
184	AGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGAACA
190	AGGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
192	GAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
193	GGRAGGA CUGAUGAGGCCGAAAGGCCGAA AAGAGGC
195	CAGGAAG CUGAUGAGGCCGAAAGGCCGAA AGAAGAG
198	GAUCAGG CUGAUGAGGCCGAAAGGCCGAA AGGAGAA
199	CGAUCA G CUGAUGAGGCCGAAAGGCCGAA AAGGAGA
205	CUGCCAC CUGAUGAGGCCGAAAGGCCGAA AUCAGGA
226	GGCAGAA CUGAUGAGGCCGAAAGGCCGAA AGCGUGG
228	CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGAGCCU
229	GCAGGCA CUGAUGAGGCCGAAAGGCCGAA AAGAGCG
243	CACUCCA CUGAUGAGGCCGAAAGGCCGAA AGUGCAG
244	UCACUCC CUGAUGAGGCCGAAAGGCCGAA AAGUGCA
253	GGGGGCG CUGAUGAGGCCGAAAGGCCGAA AUCACUC
273	CCUUGGG CUGAUGAGGCCGAAAGGCCGAA ACUCUUC
286	UUAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGUCCC
288	GAUJAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
290	CUGAUJA CUGAUGAGGCCGAAAGGCCGAA AGAGAGG
292	GGCUGAU CUGAUGAGGCCGAAAGGCCGAA AGAGAGA
295	GAGGGCU CUGAUGAGGCCGAAAGGCCGAA AUUAGAG
302	UGGGGCA CUGAUGAGGCCGAAAGGCCGAA AGGGCUG

321 AGAAGAU CUGAUGAGGCCGAAAGGCCGAA AUCUGAC
 324 UCGAGAA CUGAUGAGGCCGAAAGGCCGAA AUGAUCU
 326 GUUCGAG CUGAUGAGGCCGAAAGGCCGAA AGAUGAU
 327 GGUUCGA CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
 329 CGGGUUC CUGAUGAGGCCGAAAGGCCGAA AGAAGAU
 352 CAUGGGC CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
 361 UUGCUAC CUGAUGAGGCCGAAAGGCCGAA ACAUGGG
 364 GGUUUGC CUGAUGAGGCCGAAAGGCCGAA ACAACAU
 374 UCAGCUU CUGAUGAGGCCGAAAGGCCGAA AGGGUUU
 391 GGCACUG CUGAUGAGGCCGAAAGGCCGAA AGCUGCC
 421 UGGCCAG CUGAUGAGGCCGAAAGGCCGAA AGGGCAU
 449 AGCUGGU CUGAUGAGGCCGAAAGGCCGAA AUCUCUC
 468 GCCCCUC CUGAUGAGGCCGAAAGGCCGAA AUGGCCAC
 480 GAUGAGG CUGAUGAGGCCGAAAGGCCGAA ACAGGCC
 484 AGUAGAU CUGAUGAGGCCGAAAGGCCGAA AGGUACA
 487 CGGAGUA CUGAUGAGGCCGAAAGGCCGAA AUGAGGU
 489 CUGGGAG CUGAUGAGGCCGAAAGGCCGAA AGAUGAG
 492 GACCUUG CUGAUGAGGCCGAAAGGCCGAA AGUAGAU
 499 UGAAGAG CUGAUGAGGCCGAAAGGCCGAA ACCUGGG
 502 CCUUGAA CUGAUGAGGCCGAAAGGCCGAA AGGACCU
 504 GCCCCUUG CUGAUGAGGCCGAAAGGCCGAA AGAGGAC
 505 GGGCCUU CUGAUGAGGCCGAAAGGCCGAA AACAGGA
 525 AUGGGUG CUGAUGAGGCCGAAAGGCCGAA AGGGGCA
 538 GGGUGAG CUGAUGAGGCCGAAAGGCCGAA AGCACAU
 541 UGUGGGU CUGAUGAGGCCGAAAGGCCGAA AGGAGCA
 553 UGGCGCU CUGAUGAGGCCGAAAGGCCGAA AUGGUGU
 562 AGACGGC CUGAUGAGGCCGAAAGGCCGAA AUGGCC
 568 GGUAGGA CUGAUGAGGCCGAAAGGCCGAA ACGGCCA
 570 CUGGUAG CUGAUGAGGCCGAAAGGCCGAA AGACGGC
 573 GGUCUGG CUGAUGAGGCCGAAAGGCCGAA AGGAGAC
 586 GGAGGUU CUGAUGAGGCCGAAAGGCCGAA ACCUUGG
 592 CAGAGAG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
 595 UGGCAGA CUGAUGAGGCCGAAAGGCCGAA AGGAGGU
 597 GAUGGCA CUGAUGAGGCCGAAAGGCCGAA AGAGGAG
 604 GGCUCUU CUGAUGAGGCCGAAAGGCCGAA AUGGCAG
 637 GGGCUCA CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
 667 CCAGAUU CUGAUGAGGCCGAAAGGCCGAA AUGGCCU
 669 UCCCAGA CUGAUGAGGCCGAAAGGCCGAA AGAUGGG
 671 CCUCCCA CUGAUGAGGCCGAAAGGCCGAA AUAGAUG
 682 GCUGGAA CUGAUGAGGCCGAAAGGCCGAA ACCCCUC
 684 CAGCTGG CUGAUGAGGCCGAAAGGCCGAA AGACCCC
 685 CCAGCGU CUGAUGAGGCCGAAAGGCCGAA AAGACCC
 709 CAGCGCU CUGAUGAGGCCGAAAGGCCGAA AGUOGGU
 721 GCGGAUU CUGAUGAGGCCGAAAGGCCGAA AUCUCAG
 725 UCGGGCC CUGAUGAGGCCGAAAGGCCGAA AUUGAUC
 735 GUCGAGA CUGAUGAGGCCGAAAGGCCGAA AGUCGGG
 737 AAGUCGA CUGAUGAGGCCGAAAGGCCGAA AUAGUUG
 739 CAAAGUC CUGAUGAGGCCGAAAGGCCGAA AGAUAGU
 744 CUCGGCA CUGAUGAGGCCGAAAGGCCGAA AGUOGAG

745 ACUCGGC CUGAUGAGGCCGAAAGGCCGAA AAGUOGA
 753 CUGCCCA CUGAUGAGGCCGAAAGGCCGAA ACUCGGC
 763 CAAAGUA CUGAUGAGGCCGAAAGGCCGAA ACCUGCC
 765 CCCAAAG CUGAUGAGGCCGAAAGGCCGAA AGACCUUG
 768 GAUCCC A CUGAUGAGGCCGAAAGGCCGAA AGUJAGAC
 769 UGAUCCC CUGAUGAGGCCGAAAGGCCGAA AAGUAGA
 775 GGGCAAU CUGAUGAGGCCGAAAGGCCGAA AUCCCCA
 778 ACAGGGC CUGAUGAGGCCGAAAGGCCGAA AUGAUCC
 801 AAGGUUG CUGAUGAGGCCGAAAGGCCGAA AUGUUUC
 808 GUUUGGG CUGAUGAGGCCGAAAGGCCGAA AGGUJUGG
 809 CGUUUGG CUGAUGAGGCCGAAAGGCCGAA AAGGUUG
 820 GGCAGGG CUGAUGAGGCCGAAAGGCCGAA AGGCCGUU
 833 AUAAAAGG CUGAUGAGGCCGAAAGGCCGAA AUUUGGGG
 837 GGAAUUA CUGAUGAGGCCGAAAGGCCGAA AGGGAUU
 838 GGGUAAU CUGAUGAGGCCGAAAGGCCGAA AAGGGAU
 839 GGGGUAA CUGAUGAGGCCGAAAGGCCGAA AAAGGGAA
 841 AGGGGGU CUGAUGAGGCCGAAAGGCCGAA AUAAAAGG
 842 GAGGGGG CUGAUGAGGCCGAAAGGCCGAA AAUAAAAG
 849 UCUGAAG CUGAUGAGGCCGAAAGGCCGAA AGGGGGU
 852 GUGUCUG CUGAUGAGGCCGAAAGGCCGAA AGGAGGG
 853 GGUGUCU CUGAUGAGGCCGAAAGGCCGAA AAGGAGG
 863 ACAGGUU CUGAUGAGGCCGAAAGGCCGAA AGGGUGU
 869 GCGAGAA CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
 871 GAGCCAG CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
 872 UGAGCCA CUGAUGAGGCCGAAAGGCCGAA AAGAGGU
 878 UCUUUUU CUGAUGAGGCCGAAAGGCCGAA AGCCAGA
 890 AGCCCCC CUGAUGAGGCCGAAAGGCCGAA AUUCUCU
 898 CGACCCU CUGAUGAGGCCGAAAGGCCGAA AGCCCCC
 899 COGACCC CUGAUGAGGCCGAAAGGCCGAA AAGCCCC
 904 GGGUUCC CUGAUGAGGCCGAAAGGCCGAA ACCCUAA
 917 AAGUUCU CUGAUGAGGCCGAAAGGCCGAA AGCUUGG
 918 AAAGUUC CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
 924 UUGCUUA CUGAUGAGGCCGAAAGGCCGAA AGUUCUA
 925 GUUGCUU CUGAUGAGGCCGAAAGGCCGAA AAGUUCU
 926 UGUUGCU CUGAUGAGGCCGAAAGGCCGAA AAAGUUC
 945 GGUUUCG CUGAUGAGGCCGAAAGGCCGAA AGUGGUG
 946 AGGUUUC CUGAUGAGGCCGAAAGGCCGAA AAGUGGU
 959 AUUCCUG CUGAUGAGGCCGAAAGGCCGAA AUCCCAG
 960 CAUUCU CUGAUGAGGCCGAAAGGCCGAA AAUCCA
 1001 GAAUUCU CUGAUGAGGCCGAAAGGCCGAA AGUGGUU
 1007 CAGUUUG CUGAUGAGGCCGAAAGGCCGAA AUUCUUA
 1008 CCAGUUU CUGAUGAGGCCGAAAGGCCGAA AAUUCUU
 1021 AGUUCUG CUGAUGAGGCCGAAAGGCCGAA AGGCCCC
 1029 CCCCAAG CUGAUGAGGCCGAAAGGCCGAA AGUUCUG
 1040 AAAGCUG CUGAUGAGGCCGAAAGGCCGAA AGGCCCC
 1046 GGGAUCA CUGAUGAGGCCGAAAGGCCGAA AGCUGUA
 1047 AGGGAUU CUGAUGAGGCCGAAAGGCCGAA AAGCUGU
 1051 UGUCAGG CUGAUGAGGCCGAAAGGCCGAA AUCAAAG
 1060 GAUUCCA CUGAUGAGGCCGAAAGGCCGAA AUGUCAG

1067	GUCUCCA CUGAUGAGGCCGAAAGGCCGAA AUUCCAG
1085	AGAACCA CUGAUGAGGCCGAAAGGCCGAA AGGCUCC
1086	CAGAACC CUGAUGAGGCCGAAAGGCCGAA AAGGCUC
1090	UGGCCAG CUGAUGAGGCCGAAAGGCCGAA ACCAAAG
1091	CUGGCCA CUGAUGAGGCCGAAAGGCCGAA AACCAA
1113	UCUUCUC CUGAUGAGGCCGAAAGGCCGAA AGUCCUG
1124	UCUAGGU CUGAUGAGGCCGAAAGGCCGAA AGGUUU
1129	CAAUUUC CUGAUGAGGCCGAAAGGCCGAA AGGUGAG
1135	UUGUGUC CUGAUGAGGCCGAAAGGCCGAA AUUUCUA
1151	AAGGCCU CUGAUGAGGCCGAAAGGCCGAA AGGUCCA
1152	GAAGGCC CUGAUGAGGCCGAAAGGCCGAA AAGGUCC
1158	AGAGAGG CUGAUGAGGCCGAAAGGCCGAA AGGCCUA
1159	GAGAGAG CUGAUGAGGCCGAAAGGCCGAA AAGGCCU
1162	CUGGAGA CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
1164	AUCUGGA CUGAUGAGGCCGAAAGGCCGAA AGAGGAA
1166	ACAUUCG CUGAUGAGGCCGAAAGGCCGAA AGAGAGG
1174	GUCUGGA CUGAUGAGGCCGAAAGGCCGAA ACAUCUG
1175	AGUCUGG CUGAUGAGGCCGAAAGGCCGAA AACAUU
1176	AAGUCUG CUGAUGAGGCCGAAAGGCCGAA AAACABC
1183	CUCAGGG CUGAUGAGGCCGAAAGGCCGAA AGUCUGG
1184	UCUCAAG CUGAUGAGGCCGAAAGGCCGAA AAGUCUG
1187	GUGUCUC CUGAUGAGGCCGAAAGGCCGAA AGGAAGU
1208	CCAUGGG CUGAUGAGGCCGAAAGGCCGAA AGGGCUG
1224	AUAGAGG CUGAUGAGGCCGAAAGGCCGAA AGCUGGC
1228	AUAAAUA CUGAUGAGGCCGAAAGGCCGAA AGGGAGC
1230	ACAUTAA CUGAUGAGGCCGAAAGGCCGAA AGAGGGA
1232	AAACAU A CUGAUGAGGCCGAAAGGCCGAA AUAGAGG
1233	CAAACAU CUGAUGAGGCCGAAAGGCCGAA AUAGAG
1234	GCAAAACA CUGAUGAGGCCGAAAGGCCGAA AAAUAGA
1238	AAGUGCA CUGAUGAGGCCGAAAGGCCGAA ACAUTAA
1239	CAAGUGC CUGAUGAGGCCGAAAGGCCGAA AACAUAA
1245	UAUUCAC CUGAUGAGGCCGAAAGGCCGAA AGUGCAA
1251	AAUAAA U CUGAUGAGGCCGAAAGGCCGAA AUCACAA
1252	UAAUAAA CUGAUGAGGCCGAAAGGCCGAA AUCACA
1254	AAUAAA U CUGAUGAGGCCGAAAGGCCGAA AUAUCA
1255	AAUAAA U CUGAUGAGGCCGAAAGGCCGAA AAUAAUC
1256	UAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAAUAAU
1258	AAUAAA U CUGAUGAGGCCGAAAGGCCGAA AUAUAAU
1259	AAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAAU
1261	AUAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAUAA
1262	AAUAAA U CUGAUGAGGCCGAAAGGCCGAA AUAUAA
1263	UAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAAU
1265	AAUAAA U CUGAUGAGGCCGAAAGGCCGAA AUAUAA
1266	AAUAAA U CUGAUGAGGCCGAAAGGCCGAA AAUAAAU
1267	UAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA
1269	AAUAAA U CUGAUGAGGCCGAAAGGCCGAA AUAUAAU
1270	AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAUAAU
1272	AUAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAUAA
1273	AAUAAA U CUGAUGAGGCCGAAAGGCCGAA AAUAAAUA

1274 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1276 GUAAAUA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1277 UGUAAAU CUGAUGAGGCCGAAAGGCCGAA AUUAAA
 1278 CUGUAAA CUGAUGAGGCCGAAAGGCCGAA AAAUAAA
 1280 AUCUGUA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1281 CAUCUGU CUGAUGAGGCCGAAAGGCCGAA AUUAAA
 1282 UCAUCUG CUGAUGAGGCCGAAAGGCCGAA AAAUAAA
 1294 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA ACAUUCA
 1296 CCCAAAUA CUGAUGAGGCCGAAAGGCCGAA AUACAUU
 1297 CCCAAAU CUGAUGAGGCCGAAAGGCCGAA AUACAU
 1298 UCCCAAA CUGAUGAGGCCGAAAGGCCGAA AAAUACA
 1300 UCUCCCCA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1301 GUCUCCCC CUGAUGAGGCCGAAAGGCCGAA AUUAAA
 1315 CCCAGGA CUGAUGAGGCCGAAAGGCCGAA AC00CCGG
 1317 CCCCCAG CUGAUGAGGCCGAAAGGCCGAA AUACCCC
 1334 CAGCUCCC CUGAUGAGGCCGAAAGGCCGAA ACAUJUGG
 1345 CUGAGCC CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
 1350 CAUGUCU CUGAUGAGGCCGAAAGGCCGAA AGCCAAG
 1359 CA CGGAA CUGAUGAGGCCGAAAGGCCGAA ACAUGUC
 1360 UCACGGG CUGAUGAGGCCGAAAGGCCGAA AACAU
 1361 UUCACGG CUGAUGAGGCCGAAAGGCCGAA AAACAU
 1362 UUUCACG CUGAUGAGGCCGAAAGGCCGAA AAAACAU
 1386 AACAGCC CUGAUGAGGCCGAAAGGCCGAA AUUGUUC
 1393 ACAUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGCCU
 1394 UACAU CUGAUGAGGCCGAAAGGCCGAA AACAGCC
 1401 AGGGGGC CUGAUGAGGCCGAAAGGCCGAA ACAUGGG
 1414 AGGCACA CUGAUGAGGCCGAAAGGCCGAA AGGCCAG
 1422 UCGAAAG CUGAUGAGGCCGAAAGGCCGAA AGGCACA
 1423 AUCAAAA CUGAUGAGGCCGAAAGGCCGAA AAGGCAC
 1425 UAAUCAA CUGAUGAGGCCGAAAGGCCGAA AGAAGGC
 1426 AUAAUCA CUGAUGAGGCCGAAAGGCCGAA AAGAAGG
 1427 CAUAAUC CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
 1431 AAAACAU CUGAUGAGGCCGAAAGGCCGAA AUCAAAA
 1432 AAAAACCA CUGAUGAGGCCGAAAGGCCGAA AAUCAAA
 1436 UUUAAA CUGAUGAGGCCGAAAGGCCGAA ACATAAU
 1437 UUUAAA CUGAUGAGGCCGAAAGGCCGAA AACAU
 1438 AUUUAAA CUGAUGAGGCCGAAAGGCCGAA AAACAU
 1439 UAUUUUA CUGAUGAGGCCGAAAGGCCGAA AAAACAU
 1440 AUAUUUU CUGAUGAGGCCGAAAGGCCGAA AAAAAC
 1441 AUAUUU CUGAUGAGGCCGAAAGGCCGAA AAAAAC
 1446 CAGAUAA CUGAUGAGGCCGAAAGGCCGAA AUUUUAA
 1448 AUCAAGAU CUGAUGAGGCCGAAAGGCCGAA AUAUUUU
 1449 AAUCAGA CUGAUGAGGCCGAAAGGCCGAA AAUAU
 1451 UUAAUCA CUGAUGAGGCCGAAAGGCCGAA AUUAAU
 1456 ACAACUU CUGAUGAGGCCGAAAGGCCGAA AUCAGAU
 1457 GACPAUCU CUGAUGAGGCCGAAAGGCCGAA AAUCAGA
 1461 UUAGAC CUGAUGAGGCCGAAAGGCCGAA ACUUAU
 1464 UUGUUUA CUGAUGAGGCCGAAAGGCCGAA ACAACUU
 1466 CAUJGUU CUGAUGAGGCCGAAAGGCCGAA AGACAAC

1479 GUCACCA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
1480 GGUCACC CUGAUGAGGCCGAAAGGCCGAA AAUCAGC
1494 AAUGAGU CUGAUGAGGCCGAAAGGCCGAA ACAGUUG
1498 CAGCAAU CUGAUGAGGCCGAAAGGCCGAA AGUGACA
1501 CCUCAGC CUGAUGAGGCCGAAAGGCCGAA AUGAGUG
1512 GGGAGCA CUGAUGAGGCCGAAAGGCCGAA AGGCCUC
1517 CCCUGGG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
1528 CAGACAC CUGAUGAGGCCGAAAGGCCGAA ACUCCC
1533 GAUUACA CUGAUGAGGCCGAAAGGCCGAA ACACAAC
1537 GGCGGAU CUGAUGAGGCCGAAAGGCCGAA ACAGACA
1540 GUAGGCC CUGAUGAGGCCGAAAGGCCGAA AUUACAG
1546 UGAUAG CUGAUGAGGCCGAAAGGCCGAA AGGCCGA
1549 CACUGAA CUGAUGAGGCCGAAAGGCCGAA AGUAGGC
1551 GGCACUG CUGAUGAGGCCGAAAGGCCGAA AUAGUAG
1552 CGCCACU CUGAUGAGGCCGAAAGGCCGAA AAUAGUA
1566 CAACCUU CUGAUGAGGCCGAAAGGCCGAA AUUUCUC
1572 CCUAAGC CUGAUGAGGCCGAAAGGCCGAA ACCUUUA
1576 CUUUCU CUGAUGAGGCCGAAAGGCCGAA AGCAACC
1577 UCUUUCC CUGAUGAGGCCGAAAGGCCGAA AAGCAAC

Table 25: Mouse TNF-a HH Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
66	UgGAAAa a GcuCcA	324	GgGUGAU C GGUCCCC
101	GCGAGGU U CGUcCC	347	GAGAagU u cCCAAau
101	GCGAGgU u CuGUccC	364	CCUCcCU C UcAUCAG
102	GCAGGGUU C UgUcCCU	366	UCCUCU C AUCAGuu
102	SCAGgUU c ugUOCU	366	UcccUCU C auCAGuU
106	GUUCUgU c CCUuUCA	369	CUCUcAU C AGuuCuA
110	UgUcCCU u UCAQuCA	376	CAGuuCU a UGGGCCA
111	gUCCcUU u CaCUCAC	390	AgACCCU C AcaCuA
111	guCCQuU u CAQuCAC	396	ucaCAcU C AGAUCAU
112	UccccUuU C ACUcACU	401	cUCAGAU C AUcUUCU
116	UuUCACU C AcUGgcc	404	AGAUCAU C UUCUCAa
137	GCCaCAU C UCCeUCC	406	AUCAUCAU U CUCAAAa
139	caCAuCU C CCUCcAg	406	AUcAUcU U cUcaAAA
177	GCAUGAU C CGcGAOG	407	UCAUCUU C UCaAAau
207	AGGCaCU C CCCcAAa	409	AUCUUUCU C aAAauuC
228	GGGGQuU C CAGAACU	409	AuCuuCU c AaAAUJC
228	G3GGQuU c CAGaacU	409	aUcUUcU c AAAauUC
236	CAGaaACU C CAGGCGG	432	AGCCUGU A GCCCCAcG
236	CAGaACU c cAGgcGg		
249	GGugCCU a UgUCUcA		
249	GGuGCCU a UGucUca	444	AcGUcGU A GCAAACC
		501	AcGCCCCU C CUGGCCA
261	UCAGGCCU C UUCUCAu	560	gGgUUGU a CCUuguC
261	UCAgGCCU C UUUCUcau	560	GGguUGU A CCUugUC
263	AGCCUCU U CUCAUUC	564	UGUACCU u gUCUACU
263	AgCCUCU U CUcauUC	567	ACCUugU C UACUOCC
264	GCCUCUU C UCaAUUCC	569	CUugUCU A CUOCCAG
264	gCCUCUU C UcauUCc	572	gUCUACU C CCAGGUu
266	CUCUUCU C aUUCCUG	572	GUCUaCU c CCAGguu
269	UUCUCAU U CCUGcUU	572	GuCUaCU C CCAGGUu
270	UCUCAUU C CUGcUUuG	579	CCCAGGU u CUCUJCA
276	UCCUGcU u GUGGCAG	580	CCAGguU c UCUUcAa
297	CCACGCU C UUCUGuC	580	CCaGGGU c UCuUcaa
299	ACGCUCU U CUGuCUa	582	AGGUUCU C UUCAagg
300	CGCUCUU C UGUcUJaC	582	AGGUuCU C UUCAAGG
304	CUuCUgU c uAcUGaa	584	GUuCUCU U CAAGGGa
306	UcUGUcU. a cUGAAcU	585	UuCUCUU C AAAGGGaC
314	CUGaACU U cGGgGUG	608	CcCGaCU a CgugCUC
315	UGaACUU c GggGUGA	615	aCgUGcU C CUCAccc
315	uGaaCUU c GGGguGa	615	AcGUGGU C CUCAccc
324	gGGUGaU c GgUOCCcC	618	UGCUCUU C ACCCACA

630	ACACCGU C AGCCGau	940	GUUCUACU c cUCAGaG
630	ACACCGU C AgCCgaU	943	UACUccU C AGaGcCc
638	agcCgAU u uGCuaUc	972	UCUaaCU u AgAAAGg
643	aUUUGcU a uCUCaAa	972	ucUaaCU u AGaaAgG
645	UuGCuaU C UCaUACC	973	CUaACuU A GAAAggG
647	GCuaUCU C aUACCAG	984	AGgGgAU U auGGcuc
663	agAAaGU C AACCUCCC	984	AGGGgauU U aUGgcUc
669	UCAACC U C CUCUCUG	985	GGGgauU a uGGcUca
669	UcAACCU c cUcUCUG	997	UcAGaGU c CAACucu
672	ACCUCCU C UCUGGCCg	1010	CugUCCU c AGAgCUU
674	CUCCUCU C UGCCgUC	1017	cAGAgCU U UcAACAA
681	cUGCCgU C AagaGcC	1018	AGAgCUU U cAACAAAC
681	CUJCCgU C AACAGCC	1019	GAgCUUU c AaCpAQu
681	CUJCCgU C aaGAGcC	1073	UggGCCU c uCAuGCA
734	CCCUGGU A UGAGCCC	1096	AAGgAcU C AAAuGGG
734	CccUGGU a ugaGCCc	1106	aUGGGGcU U uccGAAu
744	AGCCCCU a UAccUGG	1107	UGGGcUU u ccGAAu
746	CCCAuAU A CCUGGGA	1108	GGgQuUU c cGaaUUC
759	GAAGAGU C uUCCAGC	1115	CcGAaAUU C ACTGGGAG
759	GAGGAGU C UUCCAGC	1133	CGAAuGu C CAuUCCU
761	GGAGUCU U CCAGCUG	1164	gagUGgU c AgGUJUGc
762	GaGCUUU C CAGCUGG	1180	UcUgUcU c agaAUGA
786	ACCAaACU C AGGCCUG	1203	aaGAuCU c AGGCCUU
798	CUGAGGU C AAUCUGC	1210	cAGGCCU U CCUaccU
802	GgUCAAU C uGCCCCaA	1211	AGGCCUU C CUaccUu
812	CCCAaAgU A cuUaGAC	1214	CCUUCCU a cCUuCAG
816	AgUAcuU a GACUUUG	1218	CcuACCU u CaGACCu
821	uUaGACU U UGCgGAG	1218	CCUaCCU U CaGACCu
822	UaGACUU U GCgGAGU	1218	cCuACCU u cAgACCu
830	GCgGAGU C cGGGCAG	1218	CCUaccU u CAGAccU
840	GGCAGGU C UACUUUG	1219	CuACCUU C AGACCUu
842	CAGGUCU A CUUUGGa	1219	CuAccUU c agACCUU
842	CAGgueU a CUUuggA	1226	CaGACCU U uCCAGAC
842	caggGuCU a CUUUgGA	1226	CAGAccU U UCCAGAC
845	GUCUACU U UGGagUC	1227	agACCUU u CCAgACu
846	UCUACUU U GGagUCA	1227	AGAccUU U CCAGACU
852	UUGGagU C AUUGCuC	1228	GAccUUU C CAGACUc
855	GagUCAU U GCuCUGU	1238	gACUcuU c CCUGAGG
887	AUCCaUU c ucUACCC	1262	CAGCCuU C CuCACaG
891	AuuucuCU a CCCaGCC	1283	CCCCccU C uaUUUAU
905	CCCaCuCU C UgaCCCC	1283	cCCCCCU C UAUUUAU
905	ccccCacU c UgACCCC	1285	ccccCUU A UUUUAu
905	CcCCACU c uGAccCC	1287	CcuCUAU u UauAUUU
914	GACCCcU U uacUCUG	1287	CCUCUAU U UAUauUU
915	ACCCCUU u acUCuGA	1288	CUCUJAUU U AUaUUUG
919	CUUUAcU c ugaCCcC	1289	UCUAUUU A UaUUUGC
928	GACCCCU u UaUugUC	1293	UUUAuUaU U UGCACUU
928	gACCCCU U UAUUguC	1293	UUUaUaU u UGcAcUu
932	CCUUUAU U guCuacU	1294	UUUAuUU U GCACUUa

1300	UUGCACU U aUuAUUu	1462	aCCuUGU u GCoUCCU
1303	CAcuUaU u AuUuAUU	1470	GccuCcU C UUUUGcU
1304	acUuAUU A UUUAUUA	1472	cuCcUCU U UUGcUUA
1306	UuAUUAU U UAUUAU	1473	uCcUCUU U UGeUUAU
1307	uAUUAUU U AUUAUUA	1474	CcUCUUU U GcUUUAUG
1307	UaUUaUU U AuuAUuU	1478	UUUUGcU U AUGUUUA
1308	AUJAUUU A UUAAUUA	1479	UUUGcUU a UGUuuAa
1310	UauUuAU U AUUAUUA	1479	UUUGcUU A UGUUUAa
1310	UADUUAU U ADUUAU	1484	UUUAUGUU U aaaAcAA
1310	UAUUUAU U AUUAUUA	1498	AAAauAU U AUCUAAc
1311	AJJUAUU A UUUAUUA	1511	AcccAaU U GUCUuAA
1311	AJJUAUU A UUUAUUA	1514	cAaUUGU C UuAAuAA
1311	AJUUAUU A UuUauUU	1516	aUUGUCU u AAuAACG
1313	UUADUUAU U UAUUAU	1529	CgcugAU u UGGUGAC
1313	UUAAUUAU U UAUUAU	1529	cGCUGAU U UGGUGAC
1313	UJAUUAU u UauUUAu	1530	gCUGAUU u gGUgacC
1314	UADUUAU U AUUAUUA	1530	GCUGAUU U GGUGACC
1314	UAUUAUU U AUUAUUA	1563	UgaAcCU c UGcUOCC
1315	AJJUAUU A UUUAUUA	1563	ugaaCCU C UGCUCCCC
1317	UAUUUAU U UAUUAU	1568	CUCUGCU C CCCAcGG
1318	AJJUAUU U AUUAUUA	1589	UGaCUGU A AUuGCC
1319	UUUAAUU A UUAAUUA	1592	CUGUAU u GcCCUAC
1326	AJJUAUU A UUUAUUA	1617	GAGAAAU A AAGaUcg
1328	UAUUUAU U UAUUUGc	1623	UAAAGaU c GCUUAAA
1329	AJJUAUU U AUUUGCu	1633	UUAAAaU a aaAAacc
1330	UUUAAUU A UUUGCuu	25	AgGgaCU a gCCaggGA
1332	UAUUUAU U UgCuuAU		
1333	AUUUAUU U gCuuAUG		
1337	auUGCU U AuGAAuG		
1338	uUUGCuu A ugAAuGu		
1346	UGAAUGU A UUUAUUA		
1348	AAUGUAU U UAUUUGG		
1349	ADGUAAU U AUUUGGa		
1350	UGUAUUU A UUUGGAA		
1352	uAUuUAU u UGGaAGG		
1352	UAUUUAU U UGGaAGG		
1353	AUUUAUU U GGaAGgC		
1369	GGGGUGU C CGGGaGG		
1398	gCuGuCU U cAGACAg		
1398	GCUGuCU U cagaCAG		
1412	GACAUGU U UUCuGUG		
1413	ACAUGUU U UCUUGUGA		
1414	CAUGUUU U CuGUGAA		
1415	ADGUUUU C uGUGAAA		
1415	ADGUUUU c UgugAaA		
1438	gaGCUGU C CCCAccU		
1451	CUUGGCCU C UcUaCCU		
1453	ggCCUCU C UaCCuUG		

Table 26: Mouse TNF- α Hammerhead Ribozyme Sequences

nt. Position	Mouse HH Ribozyme Sequence
25	UCCUGGC CUGAUGAGGCGCAAAGGCCGAA AGUCOCU
66	UGGGAGC CUGAUGAGGCGCAAAGGCCGAA AUUUCCA
101	GGGACAG CUGAUGAGGCGCAAAGGCCGAA ACCUGCC
101	GGGACAG CUGAUGAGGCGCAAAGGCCGAA ACCUGCC
102	AGGGACA CUGAUGAGGCGCAAAGGCCGAA AACCUGC
102	AGGGACA CUGAUGAGGCGCAAAGGCCGAA AACCUGC
106	UGAAAGG CUGAUGAGGCGCAAAGGCCGAA ACAGAAC
110	UGAGUGA CUGAUGAGGCGCAAAGGCCGAA AGGGACA
111	GUGAGUG CUGAUGAGGCGCAAAGGCCGAA AAGGGAC
111	GUGAGUG CUGAUGAGGCGCAAAGGCCGAA AAGGGAC
112	AGUGAGU CUGAUGAGGCGCAAAGGCCGAA AAAGGGA
115	GGCCAGU CUGAUGAGGCGCAAAGGCCGAA AGUGAAA
137	GGAGGGG CUGAUGAGGCGCAAAGGCCGAA AUUGGGC
139	CUGGAGG CUGAUGAGGCGCAAAGGCCGAA AGAUGUG
177	CGUCCGG CUGAUGAGGCGCAAAGGCCGAA AUCAUGC
207	UUUGGGG CUGAUGAGGCGCAAAGGCCGAA AGUGOCU
228	AGUUCUG CUGAUGAGGCGCAAAGGCCGAA AAGCCCC
228	AGUUCUG CUGAUGAGGCGCAAAGGCCGAA AAGCCCC
236	CCGCCUG CUGAUGAGGCGCAAAGGCCGAA AGTUCUG
236	CCGCCUG CUGAUGAGGCGCAAAGGCCGAA AGUUCUG
249	UGAGACA CUGAUGAGGCGCAAAGGCCGAA AGCCACC
249	UGAGACA CUGAUGAGGCGCAAAGGCCGAA AGCCACC
261	AUGAGAA CUGAUGAGGCGCAAAGGCCGAA AGCCUGA
261	AUGAGAA CUGAUGAGGCGCAAAGGCCGAA AGCCUGA
263	GAUGAG CUGAUGAGGCGCAAAGGCCGAA AGAGCCU
263	GAUGAG CUGAUGAGGCGCAAAGGCCGAA AGAGCCU
264	GGAAUGA CUGAUGAGGCGCAAAGGCCGAA AAGAGGC
264	GGAAUGA CUGAUGAGGCGCAAAGGCCGAA AAGAGGC
266	CAGGAUU CUGAUGAGGCGCAAAGGCCGAA AGAAGAG
269	AAGCAGG CUGAUGAGGCGCAAAGGCCGAA AUAGAGA
270	CAAGCAG CUGAUGAGGCGCAAAGGCCGAA AUAGAGA
276	CUGCCAC CUGAUGAGGCGCAAAGGCCGAA AGCAGGA
297	GACAGAA CUGAUGAGGCGCAAAGGCCGAA AGCGUGG
299	UAGACAG CUGAUGAGGCGCAAAGGCCGAA AGAGCGU
300	GUAGACA CUGAUGAGGCGCAAAGGCCGAA AAGAGCG
304	UUCAGUA CUGAUGAGGCGCAAAGGCCGAA ACAGAAAG
306	AGUUCAG CUGAUGAGGCGCAAAGGCCGAA AGACAGA
314	CAACCCG CUGAUGAGGCGCAAAGGCCGAA AGUUCAG
315	UCACCCC CUGAUGAGGCGCAAAGGCCGAA AAGUUCA

315	UCAACCCC CUGAUGAGGCCGAAAGGCCGAA AAGUUCA
324	GGGGACC CUGAUGAGGCCGAAAGGCCGAA AUCAACC
324	GGGGACC CUGAUGAGGCCGAAAGGCCGAA AUCAACC
347	AUUUGGG CUGAUGAGGCCGAAAGGCCGAA ACUUCUC
364	CGAGDGA CUGAUGAGGCCGAAAGGCCGAA AGGGAGG
366	AACUGAU CUGAUGAGGCCGAAAGGCCGAA AGAGGGA
366	AACUGAU CUGAUGAGGCCGAAAGGCCGAA AGAGGGA
369	UAGAACU CUGAUGAGGCCGAAAGGCCGAA AUGAGAG
376	UGGGCCA CUGAUGAGGCCGAAAGGCCGAA AGAACTG
390	UGAGUGU CUGAUGAGGCCGAAAGGCCGAA AGGGCCU
396	AUGAUCU CUGAUGAGGCCGAAAGGCCGAA AGGGUGA
401	AGAAGAU CUGAUGAGGCCGAAAGGCCGAA AUCCGAG
404	UUGAGAA CUGAUGAGGCCGAAAGGCCGAA AUGAUCU
406	UUUUGAG CUGAUGAGGCCGAAAGGCCGAA AGAUGAU
406	UUUUGAG CUGAUGAGGCCGAAAGGCCGAA AGAUGAU
407	AUUUUGA CUCAUUGAGGCCGAAAGGCCGAA AGAUGA
409	GAAUUUU CUGAUGAGGCCGAAAGGCCGAA AGAAGAU
409	GAAUUUU CUGAUGAGGCCGAAAGGCCGAA AGAAGAU
409	GAAUUUU CUGAUGAGGCCGAAAGGCCGAA AGAAGAU
432	CGUGGGC CUGAUGAGGCCGAAAGGCCGAA ACAGGCU
444	GGUUUGC CUGAUGAGGCCGAAAGGCCGAA AGCACGU
501	UGGCCAG CUGAUGAGGCCGAAAGGCCGAA AGGGCGU
560	GACAAGG CUGAUGAGGCCGAAAGGCCGAA ACAACCC
560	GACAAGG CUGAUGAGGCCGAAAGGCCGAA ACAACCC
564	AGUAGAC CUGAUGAGGCCGAAAGGCCGAA AGGUACA
567	GGGAGUA CUGAUGAGGCCGAAAGGCCGAA ACAAGGU
569	CUGGGAG CUGAUGAGGCCGAAAGGCCGAA AGACAAAG
572	AACCUGG CUGAUGAGGCCGAAAGGCCGAA AGUAGAC
572	AACCUGG CUGAUGAGGCCGAAAGGCCGAA AGUAGAC
572	UGAAGAG CUGAUGAGGCCGAAAGGCCGAA ACCUGGG
579	UUGAAGA CUGAUGAGGCCGAAAGGCCGAA AACCTGG
580	UUGAAGA CUGAUGAGGCCGAAAGGCCGAA AACCTGG
580	CCUUGAA CUGAUGAGGCCGAAAGGCCGAA AGAACCU
582	CCUUGAA CUGAUGAGGCCGAAAGGCCGAA AGAACCU
582	UCCCUUG CUGAUGAGGCCGAAAGGCCGAA AGAGAAC
584	GUCCCCU CUGAUGAGGCCGAAAGGCCGAA AAGAGAA
585	GAGCACG CUGAUGAGGCCGAAAGGCCGAA AGUOCCG
608	GGGUGAG CUGAUGAGGCCGAAAGGCCGAA AGCACGU
615	GGGUGAG CUGAUGAGGCCGAAAGGCCGAA AGCACGU
615	UGUGGGU CUGAUGAGGCCGAAAGGCCGAA AGGAGCA
618	AUCGGCU CUGAUGAGGCCGAAAGGCCGAA ACGGUGU
630	AUCGGCU CUGAUGAGGCCGAAAGGCCGAA ACGGUGU
630	GATAGCA CUGAUGAGGCCGAAAGGCCGAA AUCCGCU
638	UAUGAGA CUGAUGAGGCCGAAAGGCCGAA ACCAAAU
643	GGUAUGA CUGAUGAGGCCGAAAGGCCGAA AUAGCPA
645	CUGGUAU CUGAUGAGGCCGAAAGGCCGAA AGAUAGC

663 GGAGGUU CUGAUGAGGCCGAAAGGCCGAA ACUULLCU
 669 CAGAGAG CUGAUGAGGCCGAAAGGCCGAA AGGTJUGA
 669 CAGAGAG CUGAUGAGGCCGAAAGGCCGAA AGGTJUGA
 672 CGGCAGA CUGAUGAGGCCGAAAGGCCGAA AGGAGGU
 674 GACGGCA CUGAUGAGGCCGAAAGGCCGAA AGAGGAG
 681 GGCUCUU CUGAUGAGGCCGAAAGGCCGAA ACGGCAG
 681 GGCUCUU CUGAUGAGGCCGAAAGGCCGAA ACGGCAG
 681 GGCUCUU CUGAUGAGGCCGAAAGGCCGAA ACGGCAG
 734 GGGCUCA CUGAUGAGGCCGAAAGGCCGAA ACCACGG
 734 GGGCUCA CUGAUGAGGCCGAAAGGCCGAA ACCACGG
 744 CCAGGUA CUGAUGAGGCCGAAAGGCCGAA AUCCCCU
 746 UCCCAAG CUGAUGAGGCCGAAAGGCCGAA AUCCCCG
 759 GCUGGAA CUGAUGAGGCCGAAAGGCCGAA ACUCCC
 759 GCUGGAA CUGAUGAGGCCGAAAGGCCGAA ACUCCC
 761 CAGCUGG CUGAUGAGGCCGAAAGGCCGAA AGACCTC
 762 CCAGCUG CUGAUGAGGCCGAAAGGCCGAA AAGACTC
 786 CAGGCU CUGAUGAGGCCGAAAGGCCGAA AGUUGGU
 798 GCAGAUU CUGAUGAGGCCGAAAGGCCGAA ACCUCAG
 802 UGGGGCA CUGAUGAGGCCGAAAGGCCGAA AUUGACC
 812 GCUUAAG CUGAUGAGGCCGAAAGGCCGAA ACTUUGG
 816 CAAAGUC CUGAUGAGGCCGAAAGGCCGAA AAGUACJ
 821 CUCCGCA CUGAUGAGGCCGAAAGGCCGAA AGUCUUA
 822 ACUCCGC CUGAUGAGGCCGAAAGGCCGAA AAGUCUA
 830 CUGCCCG CUGAUGAGGCCGAAAGGCCGAA ACUCCGC
 840 CAAAGUA CUGAUGAGGCCGAAAGGCCGAA ACCUGCC
 842 UCCAAAG CUGAUGAGGCCGAAAGGCCGAA AGACCTG
 842 UCCAAAG CUGAUGAGGCCGAAAGGCCGAA AGACCTG
 845 GACUCCA CUGAUGAGGCCGAAAGGCCGAA AGUAGAC
 846 UGACUCC CUGAUGAGGCCGAAAGGCCGAA AAGUAGA
 852 GAGCAAU CUGAUGAGGCCGAAAGGCCGAA ACUCCAA
 855 ACAGAGC CUGAUGAGGCCGAAAGGCCGAA AUGACTC
 887 GGGUAGA CUGAUGAGGCCGAAAGGCCGAA AAUGGAU
 891 GGCUGGG CUGAUGAGGCCGAAAGGCCGAA AGAGAUU
 905 GGGGUCA CUGAUGAGGCCGAAAGGCCGAA AGUGGGG
 905 GGGGUCA CUGAUGAGGCCGAAAGGCCGAA AGUGGGG
 905 GGGGUCA CUGAUGAGGCCGAAAGGCCGAA AGUGGGG
 914 CAGAGUA CUGAUGAGGCCGAAAGGCCGAA AGGGGJC
 915 UCAGAGU CUGAUGAGGCCGAAAGGCCGAA AAAGGGGU
 919 GGGGUCA CUGAUGAGGCCGAAAGGCCGAA AGUUAAG
 928 GACAUA CUGAUGAGGCCGAAAGGCCGAA AGGGGUC
 928 GACAUA CUGAUGAGGCCGAAAGGCCGAA AGGGGUC
 932 AGUAGAC CUGAUGAGGCCGAAAGGCCGAA AUAAAGG
 940 CUCUGAG CUGAUGAGGCCGAAAGGCCGAA AGUAGAC
 943 GGGCUCU CUGAUGAGGCCGAAAGGCCGAA AGGAGUA
 972 CCUUUCU CUGAUGAGGCCGAAAGGCCGAA AGUUAGA
 972 CCUUUCU CUGAUGAGGCCGAAAGGCCGAA AGUUAGA
 973 CCCUUUC CUGAUGAGGCCGAAAGGCCGAA AAGUUAG
 984 GAGCCAU CUGAUGAGGCCGAAAGGCCGAA AUCCCCU

984 GAGCCAU CUGAUGAGGCCGAAAGGCCGAA AUCCCCU
 985 UGAGCCA CUGAUGAGGCCGAAAGGCCGAA AAUCCCC
 997 AGAGUUG CUGAUGAGGCCGAAAGGCCGAA ACUTUGA
 1010 AAGCUCU CUGAUGAGGCCGAAAGGCCGAA AGCRACAG
 1017 UUGUUGA CUGAUGAGGCCGAAAGGCCGAA AGCUCUG
 1018 GUUGUG CUGAUGAGGCCGAAAGGCCGAA AAGCUCU
 1019 AGUUGUU CUGAUGAGGCCGAAAGGCCGAA AAAGCUC
 1073 UGCAUGA CUGAUGAGGCCGAAAGGCCGAA AGGCCCA
 1096 CCCAUUU CUGAUGAGGCCGAAAGGCCGAA AGUCCUU
 1106 AUUOGGA CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
 1107 AAUUCGG CUGAUGAGGCCGAAAGGCCGAA AAGCCCA
 1108 GAUUCG CUGAUGAGGCCGAAAGGCCGAA AAAGCCC
 1115 CUCCAGU CUGAUGAGGCCGAAAGGCCGAA AAUCGG
 1133 AGGAUG CUGAUGAGGCCGAAAGGCCGAA ACAUTCG
 1164 GCAACCU CUGAUGAGGCCGAAAGGCCGAA ACCACUC
 1180 UCAIUCU CUGAUGAGGCCGAAAGGCCGAA AGCACAGA
 1203 AAGGCCU CUGAUGAGGCCGAAAGGCCGAA AGAUCUU
 1210 AGGUAGG CUGAUGAGGCCGAAAGGCCGAA AGGCCUG
 1211 AAGGUAG CUGAUGAGGCCGAAAGGCCGAA AAAGCCU
 1214 CUGAAGG CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
 1218 AGGUCUG CUGAUGAGGCCGAAAGGCCGAA AGGUAGG
 1218 AGGUCUG CUGAUGAGGCCGAAAGGCCGAA AGGUAGG
 1219 AAGGUCU CUGAUGAGGCCGAAAGGCCGAA AAGGUAG
 1219 AAGGUCU CUGAUGAGGCCGAAAGGCCGAA AAGGUAG
 1226 GUCUGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUCUG
 1226 GUCUGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUCUG
 1227 AGUCUGG CUGAUGAGGCCGAAAGGCCGAA AAGGUUC
 1227 AGUCUGG CUGAUGAGGCCGAAAGGCCGAA AAGGUUC
 1228 GAGUCUG CUGAUGAGGCCGAAAGGCCGAA AAAGGUUC
 1238 CCUCAGG CUGAUGAGGCCGAAAGGCCGAA AAGAGUC
 1262 CUGUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGCUG
 1283 ATAAAAUA CUGAUGAGGCCGAAAGGCCGAA AGGGGGG
 1283 ATAAAAUA CUGAUGAGGCCGAAAGGCCGAA AGGGGGG
 1285 ATATAAAA CUGAUGAGGCCGAAAGGCCGAA AGAGGGG
 1287 AAAAUAAA CUGAUGAGGCCGAAAGGCCGAA ATAGAGG
 1287 AAAAUAAA CUGAUGAGGCCGAAAGGCCGAA ATAGAGG
 1288 CAAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAATAGAG
 1289 GCAAUAUA CUGAUGAGGCCGAAAGGCCGAA AAATAGA
 1293 AAGUGCA CUGAUGAGGCCGAAAGGCCGAA ATATAAA
 1293 AAGUGCA CUGAUGAGGCCGAAAGGCCGAA ATATAAA
 1294 UAAGUGC CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1300 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA AGUGCAA
 1303 AAUAAA CUGAUGAGGCCGAAAGGCCGAA ATAGUG
 1304 UAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAGU
 1306 AAUAAA CUGAUGAGGCCGAAAGGCCGAA ATAAUAA
 1307 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA
 1307 AAAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUAAA

1308 UAAAUAU CUGAUGAGGCCGAAAGGCCGAA AAAUAAU
 1310 AAUAAAU CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1310 AAUAAAU CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1310 AAUAAAU CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1311 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1311 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1311 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1313 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1313 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1313 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1314 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1315 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1317 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1318 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1319 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1326 AAUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1328 GCAAATA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1329 AGCAAAU CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1330 AAGCAAA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1332 AUAGCA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1333 CAUAAGC CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1337 CAUCAU CUGAUGAGGCCGAAAGGCCGAA AGCAAAU
 1338 ACUUCA CUGAUGAGGCCGAAAGGCCGAA AAGCAAA
 1346 AAUAAA CUGAUGAGGCCGAAAGGCCGAA ACUUCA
 1348 CCAAATA CUGAUGAGGCCGAAAGGCCGAA AUACAUU
 1349 UCCAAA CUGAUGAGGCCGAAAGGCCGAA AUACAU
 1350 UCCAAA CUGAUGAGGCCGAAAGGCCGAA AAUACAU
 1352 CCTUCCA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1352 CCTUCCA CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1353 GCCUUC CUGAUGAGGCCGAAAGGCCGAA AUAAAUA
 1369 CCTCCAG CUGAUGAGGCCGAAAGGCCGAA ACACCCC
 1398 CGUCUG CUGAUGAGGCCGAAAGGCCGAA AGACAGC
 1398 CGUCUG CUGAUGAGGCCGAAAGGCCGAA AGACAGC
 1412 CACAGAA CUGAUGAGGCCGAAAGGCCGAA ACAUGUC
 1413 UCACAGA CUGAUGAGGCCGAAAGGCCGAA AACAUUG
 1414 UUCACAG CUGAUGAGGCCGAAAGGCCGAA AAACAUUG
 1415 UUUCACA CUGAUGAGGCCGAAAGGCCGAA AAAACAU
 1415 UUUCACA CUGAUGAGGCCGAAAGGCCGAA AAAACAU
 1438 AGGUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGCUC
 1451 AGGUAGA CUGAUGAGGCCGAAAGGCCGAA AGGCAG
 1453 CAAGGUA CUGAUGAGGCCGAAAGGCCGAA AGAGGCC
 1455 AACAGG CUGAUGAGGCCGAAAGGCCGAA AGAGAGG
 1462 AGGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAAGGU
 1470 AGCAAAA CUGAUGAGGCCGAAAGGCCGAA AGGAGGC
 1472 UAAGCAA CUGAUGAGGCCGAAAGGCCGAA AGAGGAG
 1473 AUAGCA CUGAUGAGGCCGAAAGGCCGAA AAGAGGA
 1474 CAUAAGC CUGAUGAGGCCGAAAGGCCGAA AAGAGGA
 1478 UAAACAU CUGAUGAGGCCGAAAGGCCGAA AGCAAAA

1479	UUAAAACA CUGAUGAGGCCGAAAGGCGGAA AAGCAAA
1479	UUAAAACA CUGAUGAGGCCGAAAGGCGGAA AAGCAAA
1484	UUGUUUU CUGAUGAGGCCGAAAGGCGGAA AACAUAA
1498	GUAGAU CUGAUGAGGCCGAAAGGCGGAA AAIAAUU
1511	UUAAGAC CUGAUGAGGCCGAAAGGCGGAA AUUGGGU
1514	UUAUUA CUGAUGAGGCCGAAAGGCGGAA ACAAUUG
1516	CGUUAUU CUGAUGAGGCCGAAAGGCGGAA AGACAAU
1529	GUCACCA CUGAUGAGGCCGAAAGGCGGAA AUCAAGG
1529	GUCACCA CUGAUGAGGCCGAAAGGCGGAA AUCAAGG
1530	GGUCACC CGGAUGAGGCCGAAAGGCGGAA AUCAAGC
1530	GGUCACC CUGAUGAGGCCGAAAGGCGGAA AUCAAGC
1563	GGGAGCA CUGAUGAGGCCGAAAGGCGGAA AGGUUCA
1563	GGGAGCA CUGAUGAGGCCGAAAGGCGGAA AGGUUCA
1568	CGGUGGG CUGAUGAGGCCGAAAGGCGGAA AGCAGAG
1589	GGGCAAU CUGAUGAGGCCGAAAGGCGGAA ACAGUCA
1592	GUAGGGC CUGAUGAGGCCGAAAGGCGGAA AUUACAG
1617	CGAUCCU CUGAUGAGGCCGAAAGGCGGAA AUUUCUC
1623	UUUAAGC CUGAUGAGGCCGAAAGGCGGAA AUUUUA
1633	GGUUUUU CUGAUGAGGCCGAAAGGCGGAA AUUUUA

Table 27: Human TNF- α Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
46	ACCCUGG AGAA GUAGU ACCAGAAAACACACGGUUGGUACAUUACUCCGUUA	ACAUACU GAC CCACGCCU
54	GAGGGGG AGAA GUGGGU ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	ACCCACG GCU CCACCCUC
185	GGAAGA AGAA GAGGA ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	UUCUCA GGC UCUUCC
201	CUGCCACG AGAA GGAGG ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	CUCUCCU GAU CGGGGAG
230	GUGGAGCA AGAA GAGCGG ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	CUCUCCU GGC UGGGGAC
234	CAAGUGG AGAA GCGCGA ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	UUCUCCU GCU GCACUCCU
254	CCUCUGGG AGAA GAUCAC ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	GUAGUCA GGC CCCAGAGA
296	GGCCAGAG AGAA GAUAGG ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	CUAUCA GGC CUCUGGCC
317	AGRAAGUG AGAA GACTGG ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	GCAGUCA GAU CAUCUCCU
387	GCACUGGG AGAA GCCCCU ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	AGGGCA GCU CCAGUGGC
404	AUTGGCCC AGAA GTUCAG ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	CUGACC GGC GGGCCAU
453	GCACCCAG AGAA GGUUAU ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	AUACCA GCU GGUGGGGCG
518	GGUGGAGG AGAA GCCUUG ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	CAAGGU GGC CCUCUCC
554	CCCGAACG AGAA GAUGGU ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	ACAUCA GGC GCAUCUCC
565	UGTGAGGA AGAA GGGAU3 ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	CAUCGC GUC UCUUACCA
576	UGACCUUAG AGAA GGUGGG ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	CCUACCA GAC CAAGGUCA
607	CTTUCUCC AGAA GGAGA ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	UCTUCCA GCU GGAGAGG
704	AGGCUUA AGAA GUCACC ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	GGUACCC GAC UGAGGU
726	GAUAGUCG AGAA GAUUGC ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	UCGAUCG GCU CGACUAC
730	UCGAGAUAG AGAA GGCCCC ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	UCGGCCG GAC UADUCUGA
824	GGGAUTGG AGAA CGGGAG ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	CUCCCU GGC CCAAUCCC
1012	GGGAUCAA AGAA GUAGCC ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	GGCUACA GCU UUGAUCC
1168	CUGGAAC AGAA GGAGAG ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	CUCUCA GAU GUUUCCG
1178	UCAAGGAA AGAA GGAAAC ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	GUUCCA GAC UUCCUUA
1202	AUGGGGAG AGAA GGGCUC ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	GAGGCCA GGC CUCCCCAU
1220	AUGAGGG AGAA GGCUC ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	GGAGCA GCU CCCUUAU
1284	AUACAUUC AGAA GUAAA ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	AUUACA GAU GAAGUAU
1340	UGACCCAA AGAA GCUCU ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	AGGAGCU GGC UGGGUCA
1390	UACRUGGG AGAA GCCUAU ACCAGAGAAACACACGGUUGGUACAUUACUCCGUUA	AUAGGU GGU CCCAUGUA

SUBSTITUTE SHEET (RULE 26)

1452	AACAUUA AGAAU ACCAGAGAAACACAGTGTGGGUA <u>C</u> UUCUCCUGUA	AUAAUCU GAU UAGUGUGU
1475	GUCACCA AGAA GCAUUG ACCAGAGAAACACAGTGTGGGUA <u>C</u> UUCUCCUGUA	CAAUCCU GAU UGGUGUGC
1513	CCCUGGG AGAA GAGGCC ACCAGAGAAACACAGTGTGGGUA <u>C</u> UUCUCCUGUA	GGCCUCU GCU CCCCGGG
1541	GAUAGUA AGAA GAUUAC ACCAGAGAAACACAGTGTGGGUA <u>C</u> UUCUCCUGUA	GUAAUCG GCC UACAUUC

Table 28: Mouse TNF- α Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
103	GUGAAGG AGAA GAGCCU ACCAGGAAACACAGGUUGGUACAUUACUUGUA	AGGUUCU GUC ACCUUCAC
256	UGGAGAGA AGAA GAGCA ACCAGGAAACACAGGUUGGUACAUUACUUGUA	UGUCUCA GGC UCUUCUA
272	CUGCCACA AGAA CGAAG ACCAGGAAACACAGGUUGGUACAUUACUUGUA	CAUUCU GGU UGGCGAG
301	GUUCAGUA AGAA GAGCG ACCAGGAAACACAGGUUGGUACAUUACUUGUA	GUUCUCA GGU UGGCGAG
325	CCUUGGG AGAA GAGAC ACCAGGAAACACAGGUUGGUACAUUACUUGUA	GUUCUCA GGU UGGCGAG
370	GCCCAUAG AGAA GAGCG ACCAGGAAACACAGGUUGGUACAUUACUUGUA	GUUCUCA GGU UGGCGAG
383	GUGUGAGG AGAA CGCCA ACCAGGAAACACAGGUUGGUACAUUACUUGUA	GUUCUCA GGU UGGCGAG
397	AGAAGAUG AGAA GAGGU ACCAGGAAACACAGGUUGGUACAUUACUUGUA	GUUCUCA GGU UGGCGAG
467	CCPACUCU AGAA GCUCCU ACCAGGAAACACAGGUUGGUACAUUACUUGUA	AACUCA GGU CAUCUUC
546	AACCCAU C AGAA GGCACC ACCAGGAAACACAGGUUGGUACAUUACUUGUA	AGGAGCA GGU GGAGGCC
549	UACAPCCC AGAA GGUCC ACCAGGAAACACAGGUUGGUACAUUACUUGUA	GUUCCCA GGC GUUCCGUU
598	GTAUCGG AGAA GGUCC ACCAGGAAACACAGGUUGGUACAUUACUUGUA	GUUCGCC GGU GGGUUGUA
603	AGCCACGU AGAA GGGAG ACCAGGAAACACAGGUUGGUACAUUACUUGUA	CAAGGUU GGC CGGACAC
631	AGCAGAAC AGAA GAGGU ACCAGGAAACACAGGUUGGUACAUUACUUGUA	CGUOCC GAC UAGGUCCU
634	GAUAGGCA AGAA GGUAG ACCAGGAAACACAGGUUGGUACAUUACUUGUA	AGGUCA GGC GUUUCGUU
675	CCUUCUAC AGAA GAGAA ACCAGGAAACACAGGUUGGUACAUUACUUGUA	GUACCC GGU UGGCAUC
691	GUCCUCUG AGAA GGGGU ACCAGGAAACACAGGUUGGUACAUUACUUGUA	GUUCUCA GGC GUUACGG
764	CCUUCUC AGAA GGAAG ACCAGGAAACACAGGUUGGUACAUUACUUGUA	AGGUCCU GGC OCAGAC
803	AGTACUCG AGAA GGUUG ACCAGGAAACACAGGUUGGUACAUUACUUGUA	UCCUCA GGU GGAGGCC
895	AGCUCCG AGAA GGGGU ACCAGGAAACACAGGUUGGUACAUUACUUGUA	UCAUUC GGC CGAGUCU
906	GUAAAGCC AGAA GAGGU ACCAGGAAACACAGGUUGGUACAUUACUUGUA	CGACCCU GGC CCACUCU
920	ADAAAGGG AGAA GAGUA ACCAGGAAACACAGGUUGGUACAUUACUUGUA	CGACCCU GGC CCUUCAC
953	AGGACACA AGAA GGGCC ACCAGGAAACACAGGUUGGUACAUUACUUGUA	UACUCU GGC CCUUCUAU
1175	CUUCUGA AGAA GGGCA ACCAGGAAACACAGGUUGGUACAUUACUUGUA	GCCCCA GUC UGGGUCCU
1220	CUUGAAGG AGAA GAGGU ACCAGGAAACACAGGUUGGUACAUUACUUGUA	UCCUCU GUC UGAGAUG
1230	AGGAGAGA AGAA GGGAG ACCAGGAAACACAGGUUGGUACAUUACUUGUA	ACCUCA GGC CCUUCAC
1256	GUAGGCAA AGAA GUCCAU ACCAGGAAACACAGGUUGGUACAUUACUUGUA	CUUCCA GAC UGUUCU
1274	UAGGAGGG AGAA GGUCCU ACCAGGAAACACAGGUUGGUACAUUACUUGUA	AGGACCA GGC CCACUUA

1393	UGUCUGAA AGAA GCUUCC ACCAGGAAACCAACGUUUCAUACAUU CAGGUGGG AGAA GCUUCG ACCAGGAAACCAACGUUUCAUACAUU
1435	CUCGUUCA AGAA GCGUUA ACCAGGAAACCAACGUUUCAUACAUU GUACGCCA AGAA GCGUUA ACCAGGAAACCAACGUUUCAUACAUU
1525	GUACGCCA AGAA GCGUUA ACCAGGAAACCAACGUUUCAUACAUU GUAGAGGC AGAA GCGUUA ACCAGGAAACCAACGUUUCAUACAUU
1542	GUAGAGGC AGAA GCGUUA ACCAGGAAACCAACGUUUCAUACAUU CGUUGGG AGAA GCGUUA ACCAGGAAACCAACGUUUCAUACAUU
1564	CGUUGGG AGAA GCGUUA ACCAGGAAACCAACGUUUCAUACAUU

Table 29: Human *bcr/abl* HH Target Sequence

Sequence ID No.	HH Target Sequence
<u><i>b2-a2</i></u> <u>Junction</u>	
20	UGACCCAUCA AUU AGGAGAGGCC
21	GUAGAAGCC CUU CAGGGGCCAGU
22	AAGAGAGCCC UUC AGGGGCCAGUA
<u><i>b3-a2</i></u> <u>Junction</u>	
23	UAAGGCAAG UUC AAAAGCCCCUUC
24	UCAAAAGCC CUU CAGGGGCCAGU
25	CAAAAGCCC UUC AGGGGCCAGUA

Table 30: Human *bcr-abl* HH Ribozyme Sequences

Sequence ID No.	HH Ribozyme Sequence
26	GGCUUCUUCU CUGAUGAGGCCGAAAGGCCGAA AUUGGAUGGUCA
27	ACUGGGCGCGUG CUGAUGAGGCCGAAAGGCCGAA AGGGCCUCUUC
28	UACUGGGCGCGU CUGAUGAGGCCGAAAGGCCGAA AAGGGCUUCUU
29	GAAGGGCUUUU CUGAUGAGGCCGAAAGGCCGAA AACUCUGCUUA
30	ACUGGGCGCGUG CUGAUGAGGCCGAAAGGCCGAA AGGGCUUUUGA
31	UACUGGGCGCGU CUGAUGAGGCCGAAAGGCCGAA AAGGGCUUUUG

Table 31: RSV (1B) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
10	GGCAAAU A AAUCAAU	276	AAAUAU A CUGAATA
14	AAUAAAU C AAUUCAG	283	ACUGAAU A CAACACA
18	AAUCAAU U CGGCCAA	295	ACAAAAAU A UGGCACU
19	AUCAUU C AGCCGAC	303	UGGCACU U UCCCTAU
54	CGAUGAU A AUACACC	304	GCCACUU U CCCUAUG
57	UGAUAAA U CACCACA	305	GCACUUU C CCTAUGC
77	UGAUGAU C ACAGACA	309	UUUCCCU A UGCCAAU
94	AGACCGU U GUACACU	317	UGCCAAU A UUCAUCA
97	CGGUUGU C ACCUGAG	319	CCAUAU U CAUCAAU
101	UGUCACU U GAGACCA	320	CAAUUU C AUCAAUC
110	AGACCAU A AUAACAU	323	UAUUCAU C AAUCAG
113	CCAUAAA U ACAUCAC	327	CAUCAAU C AUGAUGG
118	AUAACAU C ACTAACCC	337	GAUGGGU U CUUAGAA
122	CAUCACU A ACCAGAG	338	AUGGGUU C UUAGAAU
134	GAGACAU C AUAAACAC	340	GGGUUCU U AGAAUGC
137	ACAUCAU A ACACACA	341	GGUUCUU A GAAUGCA
148	CACAAAU U UAUAAUC	350	AAUGCAU U GCCAUTA
149	ACAAAUU U AUAAACU	356	UUGGCCAU U AAGCCUA
150	CAAAUUU A UAUACUU	357	UGGCCAU A AGCCUAC
152	AAUUUAU A UACUUGA	363	UAAGCCU A CAAAGCA
154	UUUUAU A CUUGAUA	372	AAAGCAU A CUCCCAU
157	AUAAACU U GAUAAA	375	GCATACU C CCAUAAU
161	ACUUGAU A AAUCAUG	380	CUCCCAU A AUAAUACA
165	GAUAAAU C AUGAUG	383	CCAUAAA U UACRAGU
176	AADGCAU A GUGAGAA	385	AUAAUUAU A CAAGUAU
188	GAAAACU U GAUGAAA	391	UACRAGU A UGAUCUC
208	GCCACAU U UACAUUC	396	GUADGAU C UCAAUCC
209	CCACAUU U ACACUCC	398	AUGAUCU C AAUCCAU
210	CACAUUU A CAUUCU	402	UCUCAAU C CAUAAA
214	UUUACAU U CCUGGUC	406	AAUCCAU A AAAUUCA
215	UUACAUU C CGGGUCA	410	CAUAAA U UCAACAC
221	UCCUGGU C AACUAG	411	AUAAAUU U CAACACA
226	GUCAACU A UGAAAG	412	UAAAUUU C AACACAA
239	UGAAACU A UUACACA	421	ACACAAU A UUCACAC
241	AAACUAA U ACACAAA	423	ACAUAU U CACACAA
242	AAUAAA U CACAAAG	424	CAUAAU C ACACAAU
251	ACAAAGU A GGAAGCA	432	ACACAAU C UAAAACA
261	AAGCACU A AAUAAA	434	ACAACU A AAACAAAC
265	ACUAAA U UAAAAAA	446	AACAACU C UAGCAU
267	UAAAUAU A AAAAUA	448	CAACUCU A UGCAUAA
274	AAAAAAU A UACUGAA	454	UAGCAU A ACUAAAC

458 CAUACAU A UACUCCA
460 UAACAU A CUCCAU
463 CUADACU C CAWAGUC
467 ACUCCAU A GUCCAGA
470 CCAUTAGU C CAGAUGG
489 UGAAAAAU U AUAGUAA
490 GAAAAAUU A UAGUAU
492 AAAUUAU A GUAUUU
495 UUATAGU A AUUAAA

Table 32: RSV (1B) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
10	AUUGAUU CUGAUGAGGCGCGAAAGGCCGA AUUUGCC
14	CUGAAUU CUGAUGAGGCCGAAAGGCCGA AUUUAUU
18	UUGGCTG CUGAUGAGGCCGAAAGGCCGA AUUGAUU
19	GUUGGCU CUGAUGAGGCCGAAAGGCCGA AAUUGAU
54	GGUGUAU CUGAUGAGGCCGAAAGGCCGA AUCAUUG
57	UGUGGUG CUGAUGAGGCCGAAAGGCCGA AUUAUCA
77	UGUCUGU CUGAUGAGGCCGAAAGGCCGA AUCAUCA
94	AAGUGAC CUGAUGAGGCCGAAAGGCCGA ACGGUCU
97	CUCAGU CUGAUGAGGCCGAAAGGCCGA ACRAAGG
101	UGGUCUC CUGAUGAGGCCGAAAGGCCGA AGUGACA
110	AUGUUAU CUGAUGAGGCCGAAAGGCCGA AUGGUCU
113	GUGAUGU CUGAUGAGGCCGAAAGGCCGA AUUAGG
118	GGUUAGU CUGAUGAGGCCGAAAGGCCGA AUGUUAU
122	CUCUGGU CUGAUGAGGCCGAAAGGCCGA AGUGAUG
134	GUGUUAU CUGAUGAGGCCGAAAGGCCGA AUGUCUC
137	UGUGUGU CUGAUGAGGCCGAAAGGCCGA AUGAUGU
148	GUAAUAU CUGAUGAGGCCGAAAGGCCGA AUUUGUG
149	AGUAIUAU CUGAUGAGGCCGAAAGGCCGA AAUUUGU
150	AAGUAIUA CUGAUGAGGCCGAAAGGCCGA AAAUUDG
152	UCAAGUA CUGAUGAGGCCGAAAGGCCGA AUAAAUU
154	UAUCAAG CUGAUGAGGCCGAAAGGCCGA AUUAAA
157	AUUUAUC CUGAUGAGGCCGAAAGGCCGA AGUAIAU
161	CAUGAUU CUGAUGAGGCCGAAAGGCCGA AUCAAGU
165	CAUCAU CUGAUGAGGCCGAAAGGCCGA AUUUAUC
176	UUCUCAC CUGAUGAGGCCGAAAGGCCGA AUGCAUU
188	UUUCAUC CUGAUGAGGCCGAAAGGCCGA AGUUUUC
208	GAUUGUA CUGAUGAGGCCGAAAGGCCGA AUGUGGC
209	GGAAUGU CUGAUGAGGCCGAAAGGCCGA AAUGUGG
210	AGGAADG CUGAUGAGGCCGAAAGGCCGA AAAUGUG
214	GACCAGG CUGAUGAGGCCGAAAGGCCGA AUGUAAA
215	UGACCAAG CUGAUGAGGCCGAAAGGCCGA AAUGUAA
221	CAUAGUU CUGAUGAGGCCGAAAGGCCGA ACCAGGA
226	CAJUUCA CUGAUGAGGCCGAAAGGCCGA AGUUGAC
239	UGUGUAA CUGAUGAGGCCGAAAGGCCGA AGUUUCA
241	UUUGUGU CUGAUGAGGCCGAAAGGCCGA AUAGUUU
242	CUUUGUG CUGAUGAGGCCGAAAGGCCGA AAUAGUU
251	UGCJUCC CUGAUGAGGCCGAAAGGCCGA ACUUUGU
261	UUAIJAUU CUGAUGAGGCCGAAAGGCCGA AGUGCUU
265	UUUUUUA CUGAUGAGGCCGAAAGGCCGA AUUUUAGU
267	UAJJUUU CUGAUGAGGCCGAAAGGCCGA AUUJJUA
274	UUCAGUA CUGAUGAGGCCGAAAGGCCGA AUUUUUU
276	UAUUCAG CUGAUGAGGCCGAAAGGCCGA AUAUUUU

283 UGUGUUG CUGAUGAGGCCGAAAGGCCGAA AUUCAGU
 295 AGUGCCA CUGAUGAGGCCGAAAGGCCGAA AUUUUGU
 303 AUAGGGG CUGAUGAGGCCGAAAGGCCGAA AGUGCCA
 304 CAUAGGG CUGAUGAGGCCGAAAGGCCGAA AAGGCC
 305 GCAGAGG CUGAUGAGGCCGAAAGGCCGAA AAAGUGC
 309 AUUGGCA CUGAUGAGGCCGAAAGGCCGAA AGGGAAA
 317 UGAUGAA CUGAUGAGGCCGAAAGGCCGAA AUUGGCA
 319 AUUGAUG CUGAUGAGGCCGAAAGGCCGAA AUAUUGG
 320 GAIUGAU CUGAUGAGGCCGAAAGGCCGAA AAUAUUG
 323 CAUGAUU CUGAUGAGGCCGAAAGGCCGAA AUGAADA
 327 CCAUCAU CUGAUGAGGCCGAAAGGCCGAA AUUGADG
 337 UUCUAG CUGAUGAGGCCGAAAGGCCGAA ACCCAUC
 338 AUUCUAA CUGAUGAGGCCGAAAGGCCGAA AACCCAU
 340 GCAUCUJ CUGAUGAGGCCGAAAGGCCGAA AGAACCC
 341 UGCAUJC CUGAUGAGGCCGAAAGGCCGAA AAGAACCC
 350 UAAUGGC CUGAUGAGGCCGAAAGGCCGAA AUGCAUJ
 356 UAGGCJJ CUGAUGAGGCCGAAAGGCCGAA AUUGCCAA
 357 GUAGGCU CUGAUGAGGCCGAAAGGCCGAA AAUGCCA
 363 UGCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGCUUA
 372 AUGGGAG CUGAUGAGGCCGAAAGGCCGAA AUGCUUU
 375 AUUAUGG CUGAUGAGGCCGAAAGGCCGAA AGUAUGC
 380 UGUAIAU CUGAUGAGGCCGAAAGGCCGAA AUGGGAG
 383 ACUUGJA CUGAUGAGGCCGAAAGGCCGAA AUUAUGG
 385 AUACUUG CUGAUGAGGCCGAAAGGCCGAA AUAAUUA
 391 GAGAUCA CUGAUGAGGCCGAAAGGCCGAA ACUUGUA
 396 GGAAUJA CUGAUGAGGCCGAAAGGCCGAA AUCAAAC
 398 ADGGAJU CUGAUGAGGCCGAAAGGCCGAA AGRUCAJ
 402 AUUUAUG CUGAUGAGGCCGAAAGGCCGAA AUUGAGA
 406 UGAAAUU CUGAUGAGGCCGAAAGGCCGAA AUGGADU
 410 GUGUUGA CUGAUGAGGCCGAAAGGCCGAA AUUUUAJ
 411 UGJGUUG CUGAUGAGGCCGAAAGGCCGAA AUUUUAU
 412 UUGUGUU CUGAUGAGGCCGAAAGGCCGAA AAUUUUA
 421 GUGUGAA CUGAUGAGGCCGAAAGGCCGAA AUUGUGU
 423 UUGUGUG CUGAUGAGGCCGAAAGGCCGAA AUAUUGU
 424 AUUGUGU CUGAUGAGGCCGAAAGGCCGAA AUUAUUG
 432 UGUUUUA CUGAUGAGGCCGAAAGGCCGAA AUUGUGU
 434 GUUGUUU CUGAUGAGGCCGAAAGGCCGAA AGAUUGU
 446 AUGCAUA CUGAUGAGGCCGAAAGGCCGAA AGUUGUU
 448 UUAUGCA CUGAUGAGGCCGAAAGGCCGAA AGAGUUG
 454 GUAUTAGU CUGAUGAGGCCGAAAGGCCGAA AUGCAUA
 458 UGGAGUA CUGAUGAGGCCGAAAGGCCGAA AGUUAUDG
 460 UAUGGAG CUGAUGAGGCCGAAAGGCCGAA AUAGUUA
 463 GACUAUG CUGAUGAGGCCGAAAGGCCGAA AGUAIAG
 467 UCUGGAC CUGAUGAGGCCGAAAGGCCGAA AUGGAGU
 470 CCAUCUG CUGAUGAGGCCGAAAGGCCGAA ACUAUGG
 489 UUACUAU CUGAUGAGGCCGAAAGGCCGAA AUUUUCA
 490 AUUACUA CUGAUGAGGCCGAAAGGCCGAA AAUUUUC
 492 AAAUJAC CUGAUGAGGCCGAAAGGCCGAA AUAAUUU
 495 UUUAAAU CUGAUGAGGCCGAAAGGCCGAA ACUAUAA

Table 33 : RSV (1C) HH target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
10	GGCAAAU A AGAATTU	165	UACAUUU A ACUAACG
16	UAAGAAU U UGAUAG	169	UUURACU A ACGCTUU
17	AAGAAUU U GAUAAGU	175	UAACGCCU U UGGCUAA
21	AUUGAU A AGUACCA	176	AACGCCU U GGCUARG
25	GAUAAGU A CCACCUA	181	UUUGGCCU A AGGCAGU
31	UACCACU U AAAUUUA	192	CAGUGAU A CAUACAA
32	ACCAUCU A AAUAAA	196	GAUACAU A CAAUCAA
36	CUUAAA U UAACUCC	201	AUACAU C AAAUUGA
37	UAAAUU U AACUCCC	206	AUCAAAU U GAADGGC
38	UAAAUUU A ACUCCC	216	AUGGCCAU U GUGUUGC
42	UUUUAUCU C CCUUGGU	221	AUUGUGU U UGUGCAU
46	ACUCCCU U GGUTAGA	222	UUGUGGU U GUCCAG
50	CCUUGGU U AGAGAUG	231	UGCAUGGU U AUUACAA
51	CUUGGUU A GAGAUGG	232	GAUGUU A UUACAG
67	CAGCAAU U CAUUGAG	234	AIGUUAU U ACAAGUA
68	AGCAAUU C AUUGAGU	235	UGUUAUU A CAGUAG
71	AAUUCAU U GAGUAUG	241	UACAAGU A GUGUAAU
76	AUUGAGU A UGAUAAA	247	UAGUCAU A UUUGCCC
81	GUAGAU A AAAGJUA	249	GUGAUAU U UGCCCCUA
87	UAAAAGU U AGAUUAC	250	UGAUAUU U GCCCUAA
88	AAAAGUU A GAAUACA	256	UGGCCU A AUAAUAA
92	GUUAGAU U ACAAAAU	259	CCCUAAU A AUAAUAU
93	UUAGAUU A CAAAAAU	262	UAAAUAU A AUAAUGU
100	ACAAAAAU U UGUUUGA	265	UAAAUAU A UUGUAGU
101	CAAAAUU U GUUGAC	267	UAAAUAU U GUAGUAA
104	AAUUGU U UGACAAU	270	AUAAUGU A GUAAAUAU
105	AUUUGUU U GCAAGUG	273	UUGUAGU A AAAUCCA
120	AUGAAGU A GCAUUGU	278	GUAAAUAU C CAAUUC
125	GUAGCAU U GUUAAA	283	AUCCAAU U UCACAAAC
128	GCAUUGU U AAAAUUA	284	CCCAAUU U CACAACA
129	CAUUGUU A AAAAUAA	285	CCPAAUU C ACAACAA
135	UAAAAAU A ACAUGCU	300	UGCCAGU A CUACAAA
143	ACAUUGCU A UACUGAU	303	CAGUACU A CAAAAUG
145	AUGCAAU A CUGAUAA	316	UGGAGGU U AUAAUAG
151	UACUGAU A AAUAAAU	317	GGAGGUU A UAUUAGG
155	GAUAAA U AAUACAU	319	AGGUUUAU A UAUGGGA
156	AUAAAUAU A AUACAUU	321	GUUAAAU A UGGGAAA
159	AAUAAA U CAUUAUA	338	AUGGAAU U AACACAU
163	AAUACAU U UACUAA	339	UGGAAUU A ACACAUU
164	AUACAUU U AACUAAAC	346	AACACAU U GCUCUCA

350 CAUUGCUC C UCAACCU
352 UUGCUCU C AACCUAA
358 UCAACCU A ADGGUCU
364 UAADGGU C UACTAGA
366 AUGGUCU A CUAGAUG
369 GUUCUACU A GAUGACA
379 UGACAAU U GUGAAAU
387 GUGAAAU U AAAUUCU
388 UGAAAUU A AAUUCUC
392 AUUAAA U CUCCAAA
393 UUAAAUU C UCCAAAA
395 AAAUUCU C CAAAAAA
405 AAAAACU A AGUGAUU
412 AAGUGAU U CAACAAU
413 AGUGAUU C AACAAAG
427 GACCAAU U AUAGGAA
428 ACCAAUU A UAUGAAU
430 CAAUUAU A UGAADCA
436 UAUGAAU C AAUUAUC
440 AAUCAAU U AUUGGAA
441 AUCAAUU A UCUGAAU
443 CAAUUAU C UGAUUA
449 UCUGAAU U ACUUGGA
450 CGUGAAU A CGUGGAU
453 AAUUACU U GGAAUUG
458 CGUGGAU U UGAUCUU
459 UUGGAU U GAUCUUA
463 AUUUGAU C UUAAUCC
465 UUGAUCU U AAUCCAU
466 UGADCUU A AUCCAU
469 UCUUAAU C CAUAAAU
473 AAUCCAU A AAUUAUA
477 CAUAAAU U AUAUUA
478 AUAAAUU A UAAUUA
480 AAAUUAU A AUUAATA
483 UUAAUUAU U AAUUAUC
484 UAUAAAUU A AUUAUCA
487 AAUUAUU A UCAACUA
489 UUAAUUAU C AACUAGC
494 AUCAACU A GCAAADC
501 AGCAAAAU C AAUGUCA
507 UCAADGU C ACUACCA
511 UGUACAU A ACACCAU
519 ACACCAU U AGUUAU
520 CACCAAU A GUUAUUA
523 CAUAGAU U AAUUA
524 AUUAGAU A AUUAUAA

Table 34: RSV (1C) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
10	AAAUCU CUGAUGACCGCGAAAGGCCGAA AUUUGCC
16	CUUAUC CUGAUGACCGCGAAAGGCCGAA AUUCUCA
17	ACUUAU CUGAUGACCGCGAAAGGCCGAA AAUUCUU
21	UGGURUCU CUGAUGACGGCGAAAGGCCGAA AUCAAU
25	UAAGUGG CUGAUGACGGCGAAAGGCCGAA ACUUAUC
31	UAAAUUU CUGAUGACGGCGAAAGGCCGAA AGGGGUA
32	UAAAUUU CUGAUGACGGCGAAAGGCCGAA AAGUGGU
36	GGAUUA CUGAUGACGGCGAAAGGCCGAA AUUUAAG
37	GGGAGUU CUGAUGACGGCGAAAGGCCGAA AUUUAAA
38	AGGGAGU CUGAUGACGGCGAAAGGCCGAA AAAUUA
42	ACCAAGG CUGAUGACGGCGAAAGGCCGAA AGUAAA
46	UCUAACC CUGAUGACGGCGAAAGGCCGAA AGGGAGU
50	CAUCUCU CUGAUGACGGCGAAAGGCCGAA ACCAAGG
51	CCAUCUC CUGAUGACGGCGAAAGGCCGAA AACCAAG
67	CUCAAUG CUGAUGACGGCGAAAGGCCGAA AUUGCUG
68	ACUCAAU CUGAUGACGGCGAAAGGCCGAA AAUUGCU
71	CAUACTC CUGAUGACGGCGAAAGGCCGAA AUGAAUU
76	UUUAUCA CUGAUGACGGCGAAAGGCCGAA ACUCAAU
81	UAACUUU CUGAUGACGGCGAAAGGCCGAA AUCAUAC
87	GUAUUCU CUGAUGACGGCGAAAGGCCGAA ACUUUUA
88	UGUAUAC CUGAUGACGGCGAAAGGCCGAA AACUUUU
92	AUUUUGU CUGAUGACGGCGAAAGGCCGAA AUCUAAC
93	AAUUUGG CUGAUGACGGCGAAAGGCCGAA AAUCAAA
100	UCAAACA CUGAUGACGGCGAAAGGCCGAA AUUUGU
101	GUCAAAC CUGAUGACGGCGAAAGGCCGAA AAUUUG
104	AUUGUCA CUGAUGACGGCGAAAGGCCGAA ACUAAUU
105	CAUUGUC CUGAUGACGGCGAAAGGCCGAA AACAAAU
120	ACAADGC CUGAUGACGGCGAAAGGCCGAA ACUCAU
125	UUUUAAC CUGAUGACGGCGAAAGGCCGAA AUCCUAC
128	UAUCCCCU CUGAUGACGGCGAAAGGCCGAA ACUADGC
129	UUAUUUU CUGAUGACGGCGAAAGGCCGAA AACAAUG
135	AGCAUGU CUGAUGACGGCGAAAGGCCGAA AUUUUUA
143	AUCAGUA CUGAUGACGGCGAAAGGCCGAA AGCAUGU
145	UUAUCAU CUGAUGACGGCGAAAGGCCGAA AUAGCAU
151	AUJAAAU CUGAUGACGGCGAAAGGCCGAA AUCAGUA
155	AUGUAUU CUGAUGACGGCGAAAGGCCGAA AUUUAUC
156	AAUGUAU CUGAUGACGGCGAAAGGCCGAA AAUUAU
159	UUAAAUG CUGAUGACGGCGAAAGGCCGAA AUUAAU
163	UUAGUUA CUGAUGACGGCGAAAGGCCGAA AUGUAU
164	GUUAGUU CUGAUGACGGCGAAAGGCCGAA AAUGUAU
165	CGUUAU CUGAUGACGGCGAAAGGCCGAA AAUDGUA

169 AAAGGCU CUGAUGAGGCCGAAAGGCCGAA AGUAAA
 175 UUAGGCA CUGAUGAGGCCGAAAGGCCGAA AGGCCUA
 176 CUGAGCC CUGAUGAGGCCGAAAGGCCGAA AAGCGUU
 181 ACUGOCU CUGAUGAGGCCGAAAGGCCGAA AGCGCAA
 192 UUGUAUG CUGAUGAGGCCGAAAGGCCGAA AUCAUG
 196 UUGAUUG CUGAUGAGGCCGAAAGGCCGAA AUGUADC
 201 UCAADUU CUGAUGAGGCCGAAAGGCCGAA AUUGUAU
 206 GCGAUUC CUGAUGAGGCCGAAAGGCCGAA AUJUGAU
 216 CAAACAC CUGAUGAGGCCGAAAGGCCGAA AUGCCAU
 221 AUGCACU CUGAUGAGGCCGAAAGGCCGAA ACACAAU
 222 CAUGCAC CUGAUGAGGCCGAAAGGCCGAA AACACAA
 231 UUGUAAU CUGAUGAGGCCGAAAGGCCGAA ACUGCA
 232 CUUGUAA CUGAUGAGGCCGAAAGGCCGAA AACAUUG
 234 UACUUGU CUGAUGAGGCCGAAAGGCCGAA ATAACAU
 235 CUACUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAACA
 241 ATAUCAC CUGAUGAGGCCGAAAGGCCGAA ACUUGUA
 247 GGGCAAA CUGAUGAGGCCGAAAGGCCGAA AUCAUCUA
 249 UAGGGCA CUGAUGAGGCCGAAAGGCCGAA ATAUCAC
 250 UUAGGGC CUGAUGAGGCCGAAAGGCCGAA AAUAAUCA
 256 UUAAUUA CUGAUGAGGCCGAAAGGCCGAA AGGGCAA
 259 AUAAUUA CUGAUGAGGCCGAAAGGCCGAA AUUAGGG
 262 ACAAAUA CUGAUGAGGCCGAAAGGCCGAA AUUAAUA
 265 ACTACAA CUGAUGAGGCCGAAAGGCCGAA AUUAAUA
 267 UUACUAC CUGAUGAGGCCGAAAGGCCGAA AUUAAUA
 270 AUUUUAC CUGAUGAGGCCGAAAGGCCGAA ACUAAU
 273 UGGAUUU CUGAUGAGGCCGAAAGGCCGAA ACUACAA
 278 GAAAUUG CUGAUGAGGCCGAAAGGCCGAA AUUUUAC
 283 GUUGUGA CUGAUGAGGCCGAAAGGCCGAA AUUUGAU
 284 UGUUGUG CUGAUGAGGCCGAAAGGCCGAA AAUUGGA
 285 UUGUUGU CUGAUGAGGCCGAAAGGCCGAA AAAUUGG
 300 UUUGUAG CUGAUGAGGCCGAAAGGCCGAA ACUGGCA
 303 CAUUUUG CUGAUGAGGCCGAAAGGCCGAA AGUACUG
 316 CAUAAAU CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
 317 CCACAUU CUGAUGAGGCCGAAAGGCCGAA AACCUCC
 319 UUCCAU CUGAUGAGGCCGAAAGGCCGAA AUUACCU
 321 UUUCCCA CGAUGAGGCCGAAAGGCCGAA AUUAAAC
 338 AUGUGUU CUGAUGAGGCCGAAAGGCCGAA AUUCCAU
 339 AAUGUGU CUGAUGAGGCCGAAAGGCCGAA AUUCCCA
 346 UGAGAGC CUGAUGAGGCCGAAAGGCCGAA AUGUGUU
 350 AGGUUGA CUGAUGAGGCCGAAAGGCCGAA AGCAAU
 352 UUAGGUU CUGAUGAGGCCGAAAGGCCGAA AGAGCAA
 358 AGACCAU CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
 364 UCUAGUA CUGAUGAGGCCGAAAGGCCGAA ACCAU
 366 CAUCUAG CUGAUGAGGCCGAAAGGCCGAA AGACCAU
 369 UGUCAUC CUGAUGAGGCCGAAAGGCCGAA AGUAGAC
 379 AUUUCAC CUGAUGAGGCCGAAAGGCCGAA AUUGUCA
 387 AGAAUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCAC
 388 GAGAAAU CUGAUGAGGCCGAAAGGCCGAA AAUUUCA
 392 UUUGGAG CUGAUGAGGCCGAAAGGCCGAA AUUUAU

393 UUUUGGA CUGAUGAGGCGGAAAGGCCGAA AAUUA
395 UUUUUG CUGAUGAGGCGGAAAGGCCGAA AGAAU
405 AAUCACU CUGAUGAGGCGGAAAGGCCGAA AGU
412 AUUGUUG CUGAUGAGGCGGAAAGGCCGAA AUCA
413 CAJUGUU CUGAUGAGGCGGAAAGGCCGAA AAUCAC
427 UCADAU CUGAUGAGGCGGAAAGGCCGAA AUJGGUC
428 AJUCADA CUGAUGAGGCGGAAAGGCCGAA AAJUGGU
430 UGADUCA CUGAUGAGGCGGAAAGGCCGAA AUAAU
436 GATAAUU CUGAUGAGGCGGAAAGGCCGAA AUUCAJA
440 UUCAGAU CUGAUGAGGCGGAAAGGCCGAA AUUGAU
441 AJUCAGA CUGAUGAGGCGGAAAGGCCGAA AAJUGAU
443 UAJJUCA CUGAUGAGGCGGAAAGGCCGAA AUAAU
449 UCCAGU CUGAUGAGGCGGAAAGGCCGAA AUUCAG
450 AUCCAAG CUGAUGAGGCGGAAAGGCCGAA AAUCAG
453 CRAAUCC CUGAUGAGGCGGAAAGGCCGAA AGUA
458 AAGAUCA CUGAUGAGGCGGAAAGGCCGAA AUCCAAG
459 UAAGAUU CUGAUGAGGCGGAAAGGCCGAA AAUCCAA
463 GGAAUAA CUGAUGAGGCGGAAAGGCCGAA AUCAA
465 AUGGAU CUGAUGAGGCGGAAAGGCCGAA AGAU
466 UAUGGAU CUGAUGAGGCGGAAAGGCCGAA AAGAU
469 AUUUAUG CUGAUGAGGCGGAAAGGCCGAA AUUA
473 UAAUAAU CUGAUGAGGCGGAAAGGCCGAA AUGGAU
477 UAAUUAU CUGAUGAGGCGGAAAGGCCGAA AUUUA
478 UUAUUAU CUGAUGAGGCGGAAAGGCCGAA AUUUA
480 UAUUAU CUGAUGAGGCGGAAAGGCCGAA AUUUA
483 UGAUAAU CUGAUGAGGCGGAAAGGCCGAA AUUUA
484 UUGAUAA CUGAUGAGGCGGAAAGGCCGAA AUUUA
487 UAGUUGA CUGAUGAGGCGGAAAGGCCGAA AUUUA
489 GCUAGUU CUGAUGAGGCGGAAAGGCCGAA AUUUA
494 GAAUUGC CUGAUGAGGCGGAAAGGCCGAA AGU
501 UGACAUU CUGAUGAGGCGGAAAGGCCGAA AUUUC
507 UGUUAGU CUGAUGAGGCGGAAAGGCCGAA ACAG
511 AUGGUGU CUGAUGAGGCGGAAAGGCCGAA AGUG
519 AJUAACU CUGAUGAGGCGGAAAGGCCGAA AUGGUG
520 UAJUAAC CUGAUGAGGCGGAAAGGCCGAA AAUGGUG
523 UUUAUAAU CUGAUGAGGCGGAAAGGCCGAA ACUA
524 UUUAUAAU CUGAUGAGGCGGAAAGGCCGAA AACUA

Table 35: RSV (N) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
9	GGCAAAU A CAAAGAU	217	GGUAUGU U AUAGGCG
21	GAUCCGU C UUAGCAA	218	GUAGUU A UAUGCCA
23	UGGCUCU U AGCRAAG	220	AUGGUUAU A UGGGAUTG
24	GGCUCUU A GCAAAGU	229	GCGAUGU C UAGGCUA
32	GCAAGU C AAGUUGA	231	GAUGUCU A GGUAAGG
37	GUCAAGU U GAAUGAU	235	UCUAGGU U AGGAAGA
45	GAAUGAU A CACUCAA	236	CUAGGUU A GGAAGAG
50	AUACACU C AACRAAG	254	ACACCAU A AAAUAC
60	CRAAGAU C AACUUCU	260	UAAAAPU A CCCAGAG
65	AUCAACU U CUGUCAU	253	AAAUACU C AGAGACG
66	UCAACUU C UGUCAUC	277	GCGGGAU A UCAUGUA
70	CUUCUGU C AUCCAGC	279	GGGAAUU C ADGJAAA
73	CUGUCAU C CAGCAA	284	AUCAGU A AARGCAA
82	AGCAAAU A CACCAUC	299	AUGGAGU A GAUGUAA
89	ACACCAU C CAACOGGA	305	UAGATGU A ACAACAC
108	AGGAGAU A GUAIUGA	315	AACACAU C GUCRACA
111	AGAUAGU A UUGUAC	318	ACAUUCU C AAGACAU
113	AUAGUAU U GUAUCUC	326	AAGACAU U AAUGGAA
117	UAUUGAU A CUCCUAA	327	AGACAUU A ADGGAAA
120	UGAUACU C CUAUUA	346	AUGAAAU U UGAAGUG
123	UACUCCU A AUUADGA	347	UGAAAUU U GAAGGUG
126	UCCUAAU U AUGAUGU	355	GAAGUGU U AACAUUG
127	CCUAAUU A UGAUGUG	356	AAGUGUU A ACATUCC
146	AACACAU C AAUAAAGU	361	UUAACAU U GGCAAGC
150	CAUCAAU A AGUUAUG	370	GCAAGCU U AACACAU
154	AAUAAAGU U AUGUGGC	371	CAAGCUU A ACACACG
155	AUAAGUU A UGUGGCA	383	CUGAAAU U CAAUCA
166	GGCAAGU U AUUAAUC	384	UGAAAUU C AAAUCAA
167	GCAUGUU A UUAAUCA	389	UUCRAAU C AACAUUG
169	AUGUUAU U AAUCACA	395	UCAACAU U GAGAATG
170	UGUJAUU A AUJACAG	401	UUGAGAU A GAAUCUA
173	UAUJAAU C ACAGAAAG	406	AUAGAAU C UAGAAAA
186	AGAUGCU A AUCAUAA	408	AGAAUCU A GAAAUC
189	UGCURAU C AUAAAUU	415	AGAAAAU C CUACAAA
192	UAAUCAU A AAUUCAC	418	AAAUCU A CAAAAAA
196	CAUAAAU U CACUGGG	431	AAAUGCU A AAAGAAA
197	AUAAAUU C ACUGGGU	449	GAGAGGU A GCUCCAG
205	ACUGGGU U AAUAGGU	453	GGUAGCU C CAGAAUA
206	CUUGGUU A AUAGGU	460	CCAGAAU A CAGGCAU
209	GGUJAAU A GGUAUGU	472	CAUGACU C UCCUGAU
213	AAUAGGU A UGUUAUA	474	UGACUCU C CUGAUG

480	UCCUGAU U GUGGGAU	696	UUUUGGU A UAGCACA
491	GGAUGAU A AUAAUAU	698	UUGGJAU A GCACAAU
494	UGAUAAU A UUAUGUA	706	GCACAAU C UUCUACC
496	AUAAUAU U AUGUAAA	708	ACAAUCU U CUACCAG
497	UAUAUAU A UGUADAG	709	CAAUUU C UACCGAGA
501	AUUAUGU A UAGCAGC	711	AUCUUCU A CCAGAGG
503	UADGUAU A GCAGCAU	726	UGGCAGU A GAGUUGA
511	GCAGCAU U AGUAAAUA	731	GUAGAGU U GAAGGGAA
512	CAGCAU U GUAAAUA	740	AAGGGAU U UUUGCAG
515	CAUAGU A AUAAACUA	741	AGGGAUU U UUGCAGG
518	UAGUAAU A ACUAAAUA	742	GGGAUUU U UGCAGGA
522	AAUACU A AAUATGC	743	GGAUUUU U GCAGGAU
526	ACUAAAUA U AGCAGCA	751	GCAGGAU U GUUUAUG
527	CUAAAUAU A GCAGCAG	754	GGAUUGU U UADGAAU
544	GACAGAU C UGGUCUU	755	GAJUGUU U AUGAAUG
549	AUCUGGU C UUACAGC	756	AUUGUUU A UGAADGC
551	CGGGUCU U ACAGCG	766	AAUGCCU A UGGUGCA
552	UGGUCUU A CAGCGGU	787	GUGAUGU U ACGGUGG
563	CGUGAU U AGGAGAG	788	UGAUGUU A CGGUGGG
564	CGUGAUU A GGAGAGC	800	GGGGAGU C UUAGCAA
573	GAGACU A AUAAUGU	802	GGAGUCU U AGCRAAA
576	AGCUAAAUAU A AUGUCCU	803	GAGUCUU A GCAAAUAU
581	AUAAUGU C CUAACAA	811	GCAAAUAU C AGUAAA
584	AUGUCCU A AAAAUG	815	AAUCAGU U AAAAUA
603	GAAAAGU U ACAAGG	816	AUCAGUU A AAAAUAU
604	AAACGUU A CAAAGGC	822	UAAAAAAU A UUAUGUU
613	AAAGGCU U ACTAACCC	824	AAAAUAU U AUGUUA
614	AAGGCUU A CUAACCA	825	AAAAUAUU A UGUUAGG
617	GCUUACU A CCCAAGG	829	AUUAUGU U AGGACAU
629	AGGACAU A GCCRACA	830	UUAUGUU A GGACAU
640	AACAGCU U CUAUGUA	840	ACAUUCU A GUGUGCA
641	ACAGCUU C UAUAGAG	866	AAACAGU U GUUGAGG
643	AGCUUCU A UGAAGUG	869	AAUOUGU U GAGGUUU
652	GAAGOGU U UGAAAAAA	875	UUGAGGU U UAUGAAU
653	AAGUGUU U GAAAAC	876	UGAGGUU U AUGAAUA
663	AAAACAU C CCCACUU	877	GAGGUUU A UGAAUAU
670	CCCACAU U UAUAGAU	883	UAUGAAU A UGCCCAA
671	CCCACUU U AUAGAUG	895	CAAAAUU U GGGUGGU
672	CCACUUU A UAGADGU	913	GCAGGAU U CUACCAU
674	ACUUAU A GAGGUUU	914	CAGGAUU C UACCAUA
680	UAGAUGU U UUUGUUC	916	GGAUUCU A CCAUATA
681	AGAUGUU U UUGUUC	921	CUACCAU A UAUUGAA
682	GAUGUUU U UGUUCAU	923	ACCAUAU A UUGAAC
683	AUGUUUU U GUUCAUU	925	CAUAAU U GAACAAC
686	UUUUUGU U CAUUUUG	943	AAAGCAU C AUUAUA
687	UUUUUGU C AUUUUGG	946	GCACAUU U AUUAUCU
690	UGUUCAU U UGGUUAU	947	CAUCAUU A UUAUCUU
691	GUUCAUU U UGGUUAU	949	UCAUUAU U AUCUUUG
692	UUCAUUAU U GGUAUAG	950	CAUUAU A UCUUUGA

952	UUAUUAU C UUUGACU
954	AUUAUCU U UGACUCA
955	UUAUCCU U GACTCAA
960	UUUGACU C AAUUC
964	ACUCAAU U UCCUCAC
965	CUCAAUU U CCUCACU
966	UCAAUU C CUCACUU
969	AUUCUCC C ACUUCUC
973	CCUCACU U CUCCAGU
974	CUCACUU C UCCAGGG
976	CACUUCU C CAGUGUA
983	CCAGUGU A GUAUUAG
986	GUGUAGU A UUAGGCA
988	GUAGUAU U AGGCAAU
989	UAGUAUU A GGCAADG
1007	CUUGGCCU A GGCAUAA
1013	UAGGCAT A AUAGGGAG
1024	GGAGAGU A CAGAGGU
1032	CAGAGGU A CACCGAG
1044	GAGGAU C AAGAUCA
1050	UCAAGAU C UAUAGUA
1052	AAGAUCAU A UAUAGUG
1054	GAUCUAU A UGAUGCA
1072	AAGGCAU A UGGUGAA
1085	AACAACU C AAAGAAA
1103	GUGUGAU U AACUACA
1104	UGUGAUU A ACCAACG
1108	AUUAACU A CAGUGUA
1115	ACAGUGU A CUAGACU
1118	GUGUACU A GACUUGA
1123	CUAGACU U GACAGCA
1139	AAGAACU A GAGGCUA
1146	AGAGGCCU A UCAAACA
1148	AGGCTAU C AAACAU
1155	CAAACAU C AGGUAAA
1160	AUCAGCU U AAUCCAA
1161	UCAGCUU A AUCCAAA
1164	GCUUAU C CAAAGA
1173	AAAAGAU A AUGAUGU
1181	AUGAUGU A GAGCUUU
1187	UAGAGCU U UGAGUUA
1188	AGAGCUU U GAGUUA
1193	UUUGAGU U AAAAAGA
1194	UUGAGUU A AUAAAAAA

Table 36: RSV (N) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
9	AUCUUUG CUGAUGAGGCCGAAAGGCCGAA AUUUGC
21	UUGCUAA CUGAUGAGGCCGAAAGGCCGAA AGCCAU
23	CUUUGCU CUGAUGAGGCCGAAAGGCCGAA AGAGCC
24	ACUUUGC CUGAUGAGGCCGAAAGGCCGAA AAGAGCC
32	UCAACUU CUGAUGAGGCCGAAAGGCCGAA ACUUUGC
37	AUCAUUC CUGAUGAGGCCGAAAGGCCGAA ACUUGAC
45	UUGAGUG CUGAUGAGGCCGAAAGGCCGAA AUCAUUC
50	CUUGUU CUGAUGAGGCCGAAAGGCCGAA AGGUUAU
60	AGAAGUU CUGAUGAGGCCGAAAGGCCGAA AUUCUUG
65	AUGACAG CUGAUGAGGCCGAAAGGCCGAA AGUUGAU
66	GAUGACA CUGAUGAGGCCGAAAGGCCGAA AAGUUGA
70	GCUGGAA CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
73	UUUGCGU CUGAUGAGGCCGAAAGGCCGAA AUGACAG
82	GAUGGGU CUGAUGAGGCCGAAAGGCCGAA AUUUGCU
89	UCCGUUG CUGAUGAGGCCGAAAGGCCGAA AUGGUGU
108	UCAAUAC CUGAUGAGGCCGAAAGGCCGAA AUCCUU
111	GUAUCAA CUGAUGAGGCCGAAAGGCCGAA ACUACU
113	GAGUACU CUGAUGAGGCCGAAAGGCCGAA AUACUAU
117	UUAGGAG CUGAUGAGGCCGAAAGGCCGAA AUCAAUA
120	UAAUUG CUGAUGAGGCCGAAAGGCCGAA AGUUAUA
123	UCAUAAU CUGAUGAGGCCGAAAGGCCGAA AGGAGUA
126	ACAUCAU CUGAUGAGGCCGAAAGGCCGAA AUUAGGA
127	CACAUCA CUGAUGAGGCCGAAAGGCCGAA AUUAGG
146	ACUUAUU CUGAUGAGGCCGAAAGGCCGAA AUGUGUU
150	CAUAAACU CUGAUGAGGCCGAAAGGCCGAA AUUGAUG
154	GCCACAU CUGAUGAGGCCGAAAGGCCGAA ACUUAUU
155	UGCCACA CUGAUGAGGCCGAAAGGCCGAA AACUUAU
166	GAUUAUU CUGAUGAGGCCGAAAGGCCGAA ACUUGCC
167	UGAUAAA CUGAUGAGGCCGAAAGGCCGAA AACAUUC
169	UGUGAUU CUGAUGAGGCCGAAAGGCCGAA AUAAACAU
170	CUGUGAU CUGAUGAGGCCGAAAGGCCGAA AUUACAC
173	CUUCUGU CUGAUGAGGCCGAAAGGCCGAA AUUAATA
186	UUAUAGAU CUGAUGAGGCCGAAAGGCCGAA AGCAUCU
189	AAUUAU CUGAUGAGGCCGAAAGGCCGAA AUUAGCA
192	GUGAAUU CUGAUGAGGCCGAAAGGCCGAA AUGAUUA
196	CCCAGUG CUGAUGAGGCCGAAAGGCCGAA AUUUAUG
197	ACCCAGU CUGAUGAGGCCGAAAGGCCGAA AAUUAU
205	ACCUUAU CUGAUGAGGCCGAAAGGCCGAA ACCCAGU
206	UACCUAU CUGAUGAGGCCGAAAGGCCGAA ACCCAG
209	ACAUACC CUGAUGAGGCCGAAAGGCCGAA AUUAACC
213	UAUAACA CUGAUGAGGCCGAAAGGCCGAA ACCUUAU

217 CCGCAUAU CUGAUGAGGCGAAAGGCGGAA ACACUACC
 218 UGGCAUUA CUGAUGAGGCGAAAGGCGGAA AACAUAC
 220 CAUCGCA CUGAUGAGGCGAAAGGCGGAA AUAAACAU
 229 UAACCUUA CUGAUGAGGCGAAAGGCGGAA ACAUCGC
 231 CCUAAACC CUGAUGAGGCGAAAGGCGGAA AGACAU
 235 UCUCUCCU CUGAUGAGGCGAAAGGCGGAA ACCUAGA
 236 CUCUUCU CUGAUGAGGCGAAAGGCGGAA ACCUAG
 254 GUAUUUU CUGAUGAGGCGAAAGGCGGAA AUGGUGU
 260 CUCUGAG CUGAUGAGGCGAAAGGCGGAA AUUUUUA
 263 CAUCUCU CUGAUGAGGCGAAAGGCGGAA AGUAUUU
 277 UACAUUA CUGAUGAGGCGAAAGGCGGAA AUCCCGC
 279 UUUACAU CUGAUGAGGCGAAAGGCGGAA AUAUCCC
 284 UUGCUUU CUGAUGAGGCGAAAGGCGGAA ACACGAAU
 299 UUACAU CUGAUGAGGCGAAAGGCGGAA ACUCCAU
 305 GUGUUGU CUGAUGAGGCGAAAGGCGGAA ACAUCUA
 315 UCUCUAC CUGAUGAGGCGAAAGGCGGAA AUGUGU
 318 AUGUCUU CUGAUGAGGCGAAAGGCGGAA ACCGAUG
 326 UUCCAUU CUGAUGAGGCGAAAGGCGGAA AUGUCUU
 327 UUUCAUU CUGAUGAGGCGAAAGGCGGAA AADGUCU
 346 CACUCA CUGAUGAGGCGAAAGGCGGAA AUUUCAU
 347 ACACUUC CUGAUGAGGCGAAAGGCGGAA AUUUCA
 355 CAUUGU CUGAUGAGGCGAAAGGCGGAA ACACUUC
 356 CCAAUUG CUGAUGAGGCGAAAGGCGGAA AACACUU
 361 GCUUGCC CUGAUGAGGCGAAAGGCGGAA AUGUCAA
 370 AGUUGUU CUGAUGAGGCGAAAGGCGGAA AGCUUGC
 371 CAGUUGU CUGAUGAGGCGAAAGGCGGAA AAGCUUG
 383 UGAAUUG CUGAUGAGGCGAAAGGCGGAA AUUUCAG
 384 UUGAUUU CUGAUGAGGCGAAAGGCGGAA AAUUUCA
 389 CAUUGUU CUGAUGAGGCGAAAGGCGGAA AUUUGAA
 395 CUACUCU CUGAUGAGGCGAAAGGCGGAA AUGUUGA
 401 UAGAUUC CUGAUGAGGCGAAAGGCGGAA AUUCCAA
 406 UUUUCUA CUGAUGAGGCGAAAGGCGGAA AUUUCAU
 408 GAUUUUC CUGAUGAGGCGAAAGGCGGAA AGAUUCU
 415 UUUGUAG CUGAUGAGGCGAAAGGCGGAA AUUUUCU
 418 UUUUUUG CUGAUGAGGCGAAAGGCGGAA AGGAAU
 431 UUUCUUU CUGAUGAGGCGAAAGGCGGAA AGCAUJJ
 449 CUGGAGC CUGAUGAGGCGAAAGGCGGAA ACCUCUC
 453 UAUUCUG CUGAUGAGGCGAAAGGCGGAA AGCUACC
 460 AUGCCUG CUGAUGAGGCGAAAGGCGGAA AUUCUGG
 472 AUCAGGA CUGAUGAGGCGAAAGGCGGAA AGUCAUG
 474 CAAUCAG CUGAUGAGGCGAAAGGCGGAA AGAGUCA
 480 AUCCCAC CUGAUGAGGCGAAAGGCGGAA AUCCAGGA
 491 AUAAUAU CUGAUGAGGCGAAAGGCGGAA AUCAUCC
 494 UACAUAA CUGAUGAGGCGAAAGGCGGAA AUUAUCA
 496 UAUACAU CUGAUGAGGCGAAAGGCGGAA AUAAUAU
 497 CUUACAU CUGAUGAGGCGAAAGGCGGAA AUAAUAU
 501 GCUGCUA CUGAUGAGGCGAAAGGCGGAA ACUAAAU
 503 AUGUGC CUGAUGAGGCGAAAGGCGGAA AUACAU
 511 UAUUACU CUGAUGAGGCGAAAGGCGGAA AUGCUGC

512 UUAUUAAC CUGAUGAGCCCGAAAGGCCGAA AAUGCUG
 515 UAGUUAU CUGAUGAGCCCGAAAGGCCGAA ACUUAUG
 518 AUUUAGU CUGAUGAGCCCGAAAGGCCGAA AUUACUA
 522 GCUAAUU CUGAUGAGCCCGAAAGGCCGAA AGUUAUU
 526 UGCUGCU CUGAUGAGCCCGAAAGGCCGAA AUUAGU
 527 CUGCUGC CUGAUGAGCCCGAAAGGCCGAA AUUUAG
 544 AAGACCA CUGAUGAGCCCGAAAGGCCGAA AUCUGUC
 549 CGUGUAA CUGAUGAGCCCGAAAGGCCGAA ACCAGAU
 551 CGGCUGU CUGAUGAGCCCGAAAGGCCGAA AGACCAG
 552 AOGGCGU CUGAUGAGCCCGAAAGGCCGAA AAGACCA
 563 CUCUCCU CUGAUGAGCCCGAAAGGCCGAA AUCAAGG
 564 GCGUCUC CUGAUGAGCCCGAAAGGCCGAA AAUCACG
 573 ACAUUAU CUGAUGAGCCCGAAAGGCCGAA AGCUCUC
 576 AGGACAU CUGAUGAGCCCGAAAGGCCGAA AUUAGCU
 581 UUUUAG CUGAUGAGCCCGAAAGGCCGAA ACAUUAU
 584 CAUJJUU CUGAUGAGCCCGAAAGGCCGAA AGGACAU
 603 CCJJJUG CUGAUGAGCCCGAAAGGCCGAA ACGUUUC
 604 GCGJJUG CUGAUGAGCCCGAAAGGCCGAA AACGUUU
 613 GGGUAGU CUGAUGAGCCCGAAAGGCCGAA AGCCUUU
 614 UGGGUAG CUGAUGAGCCCGAAAGGCCGAA AAGCCUU
 617 CCGUUGGG CUGAUGAGCCCGAAAGGCCGAA AGUAAGC
 629 UGUUGGC CUGAUGAGCCCGAAAGGCCGAA AUGUCCU
 640 UUCATAG CUGAUGAGCCCGAAAGGCCGAA AGCUUU
 641 CUUCATA CUGAUGAGCCCGAAAGGCCGAA AAGCUGU
 643 CACUUCA CUGAUGAGCCCGAAAGGCCGAA AGAAGCU
 652 UUUUUCA CUGAUGAGCCCGAAAGGCCGAA ACACUUC
 653 GUUUUUC CUGAUGAGCCCGAAAGGCCGAA AACACUU
 663 AAGUGGG CUGAUGAGCCCGAAAGGCCGAA AUGUUUU
 670 AUCUATA CUGAUGAGCCCGAAAGGCCGAA AGUGGGG
 671 CAUCUAU CUGAUGAGCCCGAAAGGCCGAA AAGUGGG
 672 ACAUCUA CUGAUGAGCCCGAAAGGCCGAA AAAGUGG
 674 AAACAUU CUGAUGAGCCCGAAAGGCCGAA AUAAAGU
 680 GAACAAA CUGAUGAGCCCGAAAGGCCGAA ACRUCUA
 681 UGAACAA CUGAUGAGCCCGAAAGGCCGAA AACADCU
 682 AUGAACA CUGAUGAGCCCGAAAGGCCGAA AAACAUC
 683 AAUGAAC CUGAUGAGCCCGAAAGGCCGAA AAAACAU
 686 CAAAAUG CUGAUGAGCCCGAAAGGCCGAA AAAAAAA
 687 CCAAAAU CUGAUGAGCCCGAAAGGCCGAA AACAAAA
 690 AUACCAA CUGAUGAGCCCGAAAGGCCGAA AUGAACA
 691 UAUACCA CUGAUGAGCCCGAAAGGCCGAA AAUGAAC
 692 CUAUACC CUGAUGAGCCCGAAAGGCCGAA AAAUGAA
 696 UGUGCUA CUGAUGAGCCCGAAAGGCCGAA ACCAAAA
 698 AUUGUGC CUGAUGAGCCCGAAAGGCCGAA AUACCAA
 706 GGUAGAA CUGAUGAGCCCGAAAGGCCGAA AUUGUGC
 708 CUGGUAG CUGAUGAGCCCGAAAGGCCGAA AGAUUGU
 709 UCUGGUA CUGAUGAGCCCGAAAGGCCGAA AAGAUUG
 711 CCUCUGG CUGAUGAGCCCGAAAGGCCGAA AGAAGAU
 726 UCAACUC CUGAUGAGCCCGAAAGGCCGAA ACUGCCA
 731 UCCCUUC CUGAUGAGCCCGAAAGGCCGAA ACUCUAC

740 CUGCAAA CUGAUGAGGCCGAAAGGCCGAA AUCCCCU
 741 CCUGCAA CUGAUGAGGCCGAAAGGCCGAA AAUCCCU
 742 UCCUGCA CUGAUGAGGCCGAAAGGCCGAA AAAUCCC
 743 AUCCUGC CUGAUGAGGCCGAAAGGCCGAA AAAAUCC
 751 CAUAAAC CUGAUGAGGCCGAAAGGCCGAA AUCCUGC
 754 AUUCAUA CUGAUGAGGCCGAAAGGCCGAA ACAAUCC
 755 CAUUCAU CUGAUGAGGCCGAAAGGCCGAA AACAAUC
 756 GCAUUCA CUGAUGAGGCCGAAAGGCCGAA AACAAAU
 765 UGCACCA CUGAUGAGGCCGAAAGGCCGAA AGGCAUU
 787 CCACCGU CUGAUGAGGCCGAAAGGCCGAA ACAUCAC
 788 CCCACCG CUGAUGAGGCCGAAAGGCCGAA AACACCA
 800 UUGCUAA CUGAUGAGGCCGAAAGGCCGAA ACUCCCC
 802 UUUUGCU CUGAUGAGGCCGAAAGGCCGAA AGACUCC
 803 AUUUUGC CUGAUGAGGCCGAAAGGCCGAA AAGACUC
 811 UUUUACU CUGAUGAGGCCGAAAGGCCGAA AUUUUGC
 815 UAUUUUU CUGAUGAGGCCGAAAGGCCGAA ACUGAUU
 816 AUUUUUU CUGAUGAGGCCGAAAGGCCGAA AACUGAU
 822 AACAUAA CUGAUGAGGCCGAAAGGCCGAA AUUUUUA
 824 CUAACAU CUGAUGAGGCCGAAAGGCCGAA ADAUUUU
 825 CCTAACAC CUGAUGAGGCCGAAAGGCCGAA AAUAAUU
 829 AUGUCCU CUGAUGAGGCCGAAAGGCCGAA ACUAAAU
 830 CAUGUCC CUGAUGAGGCCGAAAGGCCGAA AACAUAA
 840 UGCACAC CUGAUGAGGCCGAAAGGCCGAA AGCAGGU
 866 CCUCAAC CUGAUGAGGCCGAAAGGCCGAA ACUUGUU
 869 AAACCUC CUGAUGAGGCCGAAAGGCCGAA ACUACUU
 875 AUUCAUA CUGAUGAGGCCGAAAGGCCGAA ACCUCAA
 876 UAUUCAU CUGAUGAGGCCGAAAGGCCGAA AACCUCA
 877 AUAUUCA CUGAUGAGGCCGAAAGGCCGAA AAACCUC
 883 UUGGGCA CUGAUGAGGCCGAAAGGCCGAA AUUCAUA
 895 ACCACCC CUGAUGAGGCCGAAAGGCCGAA AUUUUUG
 913 AUGGUAG CUGAUGAGGCCGAAAGGCCGAA AUCCUGC
 914 UAUJGUA CUGAUGAGGCCGAAAGGCCGAA AAUCCUG
 916 UAUJUAG CUGAUGAGGCCGAAAGGCCGAA AGAAUCC
 921 UUCAUA CUGAUGAGGCCGAAAGGCCGAA AUGGUAG
 923 UGUUCAA CUGAUGAGGCCGAAAGGCCGAA AUAUUGU
 925 GUUGUUC CUGAUGAGGCCGAAAGGCCGAA AUAUADG
 943 UAAUAAU CUGAUGAGGCCGAAAGGCCGAA AUGUUU
 946 AGAUAAU CUGAUGAGGCCGAAAGGCCGAA AUGAUGC
 947 AAGAUAA CUGAUGAGGCCGAAAGGCCGAA AAUGAUG
 949 CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AUAAUGA
 950 UCAAAGA CUGAUGAGGCCGAAAGGCCGAA AUAAUG
 952 AGUAAA CUGAUGAGGCCGAAAGGCCGAA AUAAUAA
 954 UGAGUCA CUGAUGAGGCCGAAAGGCCGAA AGAUAAU
 955 UUGAGUC CUGAUGAGGCCGAAAGGCCGAA AAGAUAA
 960 CGAAAUU CUGAUGAGGCCGAAAGGCCGAA AGUCAAA
 964 GUGAGGA CUGAUGAGGCCGAAAGGCCGAA AUUGAGU
 965 AGUGAGG CUGAUGAGGCCGAAAGGCCGAA AAUUGAG
 966 AAGUGAG CUGAUGAGGCCGAAAGGCCGAA AAUUGUA
 969 GAGAAGU CUGAUGAGGCCGAAAGGCCGAA AGGAAAU

973 ACUGGAG CUGAUGAGGCCGAAAGGCCGAA AGUGAGG
974 CACUGGA CUGAUGAGGCCGAAAGGCCGAA AAGCGAG
976 UACACUG CUGAUGAGGCCGAAAGGCCGAA AGAACUG
983 CUAUAC CUGAUGAGGCCGAAAGGCCGAA ACACUUG
986 UGCCUA CUGAUGAGGCCGAAAGGCCGAA ACUACAC
988 AUUGCUC CUGAUGAGGCCGAAAGGCCGAA AUACUAC
989 CAUUGCC CUGAUGAGGCCGAAAGGCCGAA AUACUAC
1007 UUAUGCC CUGAUGAGGCCGAAAGGCCGAA AGGCCAG
1013 CUCCCCAU CUGAUGAGGCCGAAAGGCCGAA AUGCCUA
1024 ACCUCUG CUGAUGAGGCCGAAAGGCCGAA ACUCUCC
1032 CUCGGUG CUGAUGAGGCCGAAAGGCCGAA ACCUCUG
1044 AGAUCUU CUGAUGAGGCCGAAAGGCCGAA AUUCUC
1050 UCAUATA CUGAUGAGGCCGAAAGGCCGAA AUUCUGA
1052 CAUCAUA CUGAUGAGGCCGAAAGGCCGAA AGAUCUU
1054 UGCAUCA CUGAUGAGGCCGAAAGGCCGAA AUACAC
1072 UUCAGCA CUGAUGAGGCCGAAAGGCCGAA AUGCCUU
1085 UUUUUUU CUGAUGAGGCCGAAAGGCCGAA AGUUGUU
1103 UGUAGUU CUGAUGAGGCCGAAAGGCCGAA AUCAAC
1104 CUGUAGU CUGAUGAGGCCGAAAGGCCGAA AAUCACA
1108 UACACUG CUGAUGAGGCCGAAAGGCCGAA AGUUAU
1115 AGUCUAG CUGAUGAGGCCGAAAGGCCGAA ACACUG
1118 UCAAGUC CUGAUGAGGCCGAAAGGCCGAA AGUACAC
1123 UGCUGUC CUGAUGAGGCCGAAAGGCCGAA AGUCUAG
1139 UAGCCUC CUGAUGAGGCCGAAAGGCCGAA AGUUCUU
1146 UGUUUGA CUGAUGAGGCCGAAAGGCCGAA AGCCUCU
1148 GAUGUUU CUGAUGAGGCCGAAAGGCCGAA AUAGCCU
1155 UUAAGCU CUGAUGAGGCCGAAAGGCCGAA AGUUUG
1160 UUGGAAU CUGAUGAGGCCGAAAGGCCGAA ACCUGAU
1161 UUUUGAU CUGAUGAGGCCGAAAGGCCGAA AAGCUGA
1164 UCUCUUU CUGAUGAGGCCGAAAGGCCGAA AUUAAGC
1173 ACAUCAU CUGAUGAGGCCGAAAGGCCGAA AUUCUUU
1181 AAAGCUC CUGAUGAGGCCGAAAGGCCGAA ACACUAC
1187 UAACUCA CUGAUGAGGCCGAAAGGCCGAA AGCUCUA
1188 UUAACUC CUGAUGAGGCCGAAAGGCCGAA AAGCUC
1193 UUUUAAU CUGAUGAGGCCGAAAGGCCGAA ACUCUAA
1194 UUUUUAAU CUGAUGAGGCCGAAAGGCCGAA AACUCAA

Table 87: RSV (1B) HP Ribozyme/Substrate Sequence

nt. Position	HP Ribozyme Sequence	Substrate
70	CUGUUC AGAA GUCCU ACCAGAGAACCAACCUUUGGUCAUUCUGGU	AAAAGCU GAU GUUCGCG
91	CUUGUUC AGAA GUCCU ACCAGAGAACCAACCUUUGGUCAUUCUGGU	UAGGACC GUU GUUCGCG
472	CGGCCUCC AGAA GCAAU ACCAGAGAACCAACCUUUGGUCAUUCUGGU	UAGUCCU GAU GUUCGCG

Table 38: RSV (N) HP Ribozyme/Substrate Sequence

nt. Position	Hairpin Ribozyme Sequence	Substrate
476	AUCCCAUA AGAA GGGAG ACCAGGAAACACGUUCGUUGGGUACGUUCCGUU	CUCUCCU GAA UGUUGAU
540	AGGACCGG AGAA GUCCC ACCAGGAAACACGUUCGUUGGGUACGUUCCGUU	GGGGAAU GAA CUGGUUU
554	CUAAAAC AGAA GUAAA ACCAGGAAACACGUUCGUUGGGUACGUUCCGUU	UCUUAUA GCC GUAGUUA
636	UUCUUNGA AGAA GUAGC ACCAGGAAACACGUUCGUUGGGUACGUUCCGUU	GCCUACH GCU UCUUAGA
998	CCUUGGCC AGAA GGUUA ACCAGGAAACACGUUCGUUGGGUACGUUCCGUU	CAUUCU GCU GGCCUAGG
1156	UUGGUUA AGAA GUUGUU ACCAGGAAACACGUUCGUUGGGUACGUUCCGUU	AGCUAUA GCU UAUUCCUA

SUBSTITUTE SHEET (RULE 26)

Table 39: Large-Scale Synthesis

Sequence	Activator [Added/Final] (min)	Amidite [Added/Final] (min)	Time*	% Full Length Product
A ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	85
A ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	89
(GGU) ₃ GGT	T [0.50/0.33]	[0.1/0.02]	15 m	78
(GGU) ₃ GGT	S [0.25/0.17]	[0.1/0.02]	15 m	81
C ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	90
C ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	97
U ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	80
U ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	85
A (36-mer)	T [0.50/0.33]	[0.1/0.02]	15/15m	21
A (36-mer)	S [0.25/0.17]	[0.1/0.02]	15/15 m	25
A (36-mer)	S [0.50/0.24]	[0.1/0.03]	15/15 m	25
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	15/15 m	38
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	10/5 m	42

*Where two coupling times are indicated the first refers to RNA coupling and the second to 2'-O-methyl coupling. S = 5-S-Ethyltetrazole, T = tetrazole activator. A is 5' -ucu ccA UCU GAU GAG GCC GAA AGG CCG AAA Auc ccu -3' where lowercase represents 2'-O-methylnucleotides.

Table 40: Base Deprotection

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
iBu(GGU) ₄	NH ₄ OH/EtOH	16 h	55	62.5
	MA	10 m	65	62.7
	AMA	10 m	65	74.8
	MA	10 m	55	75.0
	AMA	10 m	55	77.2
iPrP(GGU) ₄	NH ₄ OH/EtOH	4 h	65	44.8
	MA	10 m	65	65.9
	AMA	10 m	65	59.8
	MA	10 m	55	61.3
	AMA	10 m	55	60.1
C ₉ U	NH ₄ OH/EtOH	4 h	65	75.2
	MA	10 m	65	79.1
	AMA	10 m	65	77.1
	MA	10 m	55	79.8
	AMA	10 m	55	75.5
A (36-mer)	NH ₄ OH/EtOH	4 h	65	22.7
	MA	10 m	65	28.9

Table 41: 2'-O-Alkylsilyl Deprotection

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
A ₉ T	TBAF	24 h	20	84.5
	1.4 M HF	0.5 h	65	81.0
(GGU) ₄	TBAF	24 h	20	60.9
	1.4 M HF	0.5 h	65	67.8
C ₁₀	TBAF	24 h	20	86.2
	1.4 M HF	0.5 h	65	86.1
U ₁₀	TBAF	24 h	20	84.8
	1.4 M HF	0.5 h	65	84.5
B (36-mer)	TBAF	24 h	20	25.2
	1.4 M HF	1.5 h	65	30.6
A (36-mer)	TBAF	24 h	20	29.7
	1.4 M HF	1.5 h	65	30.4

B is 5'- UCU CCA UCU GAU GAG GCC GAA AGG CCG AAA AUC CCU
-3'.

**Table 42: NMR Data for UC Dimers containing
Phosphorothioate Linkage**

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3524	ribo	2 x 3 s	10.4	2 x 100 s	95.9
3525	ribo	2 x 3 s	10.4	2 x 75 s	92.6
3530	ribo	2 x 3 s	10.4	2 x 75 s	92.1
3526	ribo	1 x 5 s	08.6	1 x 300 s	100.0
3578	ribo	1 x 5 s	08.6	1 x 250 s	100.0
3529	ribo	1 x 5 s	08.6	1 x 150 s	73.7

**Table 43: NMR Data for 15-mer RNA containing
Phosphorothioate Linkages**

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3581	ribo	1 x 5 s	08.6	1 x 250 s	99.6
3663	ribo	2 x 4 s	13.8	2 x 300 s	100.0
3582	2'-O-Me	1 x 5 s	08.6	1 x 250 s	99.7
3668	2'-O-Me	2 x 4 s	13.8	2 x 300 s	99.8
3682	2'-O-Me	1 x 5 s	08.6	1 x 300 s	99.8

Table 44. Kinetics of Self-Processing *In Vitro*

Self-Processing Constructs	$k (\text{min}^{-1})^*$
HH	1.16 ± 0.08
HDV	0.56 ± 0.15
HP(GC)	0.36 ± 0.06
HP(GU)	0.054 ± 0.003

* k represents the unimolecular rate constant for ribozyme self-cleavage determined from a non-linear, least-squares fit (KaleidaGraph, Synergy Software, Reeding, PA) to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt} (1 - e^{-kt})$$

The equation describes the extent of ribozyme processing in the presence of ongoing transcription (Long & Uhlenbeck, 1994 *Proc. Natl. Acad. Sci. USA* 91, 6977) as a function of time (t) and the unimolecular rate constant for cleavage (k). Each value of k represents the average (\pm range) of values determined from two experiments.

Table 45

Entry	Modification	$t_{1/2}$ (m) Activity (t_A)	$t_{1/2}$ (m) Stability (t_S)	$\beta = t_S/t_A$ $\times 10$
1	U4 & U7 = U	1	0.1	1
2	U4 & U7 = 2'-O-Me-U	4	260	650
3	U4 = 2'=CH ₂ -U	6.5	120	180
4	U7 = 2'=CH ₂ -U	8	280	350
5	U4 & U7 = 2'=CH ₂ -U	9.5	120	130
6	U4 = 2'=CF ₂ -U	5	320	640
7	U7 = 2'=CF ₂ -U	4	220	550
8	U4 & U7 = 2'=CF ₂ -U	20	320	160
9	U4 = 2'-F-U	4	320	800
10	U7 = 2'-F-U	8	400	500
11	U4 & U7 = 2'-F-U	4	300	750
12	U4 = 2'-C-Allyl-U	3	>500	>1700
13	U7 = 2'-C-Allyl-U	3	220	730
14	U4 & U7 = 2'-C-Allyl-U	3	120	400
15	U4 = 2'-araF-U	5	>500	>1000
16	U7 = 2'-araF-U	4	350	875
17	U4 & U7 = 2'-araF-U	15	500	330
18	U4 = 2'-NH ₂ -U	10	500	500
19	U7 = 2'-NH ₂ -U	5	500	1000
20	U4 & U7 = 2'-NH ₂ -U	2	300	1500
21	U4 = dU	6	100	170
22	U4 & U7 = dU	4	240	600

CLAIMSWhat is claimed is:

1. An enzymatic nucleic acid molecule which cleaves ICAM-1 mRNA, IL-5 mRNA, *rel A* mRNA, TNF- α mRNA sites shown in Table 23, 25, 27, or 28, CML associated mRNA selected from those identified as SEQ. ID NOS 1-25, or RSV mRNA or RSV genomic RNA in a region selected from the group consisting of 1C, 1B and N.
2. The enzymatic nucleic acid molecule of claim 1, the binding arms of which contain sequences complementary to any one of the sequences defined in any of those in Tables 2, 3, 6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36, and 37.
3. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said nucleic acid molecule is in a hammerhead motif.
4. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said RNA molecule is in a hairpin, hepatitis delta virus, group 1 intron, *Neurospora* VS RNA or RNaseP RNA motif.
5. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 12 and 100 bases complementary to said mRNA or genomic RNA.
- 20 6. The enzymatic nucleic acid molecule of claim 5 comprising between 14 and 24 bases complementary to said mRNA or genomic RNA.
7. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 5 and 23 bases complementary to said mRNA or genomic RNA.
- 25 8. The enzymatic nucleic acid molecule of claim 7 comprising between 10 and 18 bases complementary to said mRNA or genomic RNA.
9. An enzymatic nucleic acid molecule consisting essentially of a sequence selected from the group of those shown in Tables 4-8, 10, 12, 14-16, 19-22, 24, 26-28, 30, 32, 34 and 36-38.
- 30 10. A mammalian cell including an enzymatic nucleic acid molecule of claims 1 or 2.

11. The cell of claim 10, wherein said cell is a human cell.
12. An expression vector including nucleic acid encoding an enzymatic nucleic acid molecule or multiple enzymatic molecules of claims 1 or 2 in a manner which allows expression of that enzymatic RNA molecule(s) within a mammalian cell.
5
13. A mammalian cell including an expression vector of claim 12.
14. The cell of claim 13, wherein said cell is a human cell.
15. A method for treatment of a pathological condition related to the mRNA level of ICAM-1, IL-5, *rel A*, TNF- α , or RSV by administering to a patient an enzymatic nucleic acid molecule of claim 1 or 2.
10
16. A method for treatment of a pathological condition related to the mRNA level of ICAM-1, IL-5, *rel A*, TNF- α , or RSV by administering to a patient an expression vector of claim 12.
17. The method of claims 15 or 16, wherein said patient is a human.
15
18. The method of claim 17 wherein said condition is selected from the group consisting of atherosclerosis, myocardial infarction, stroke, restenosis, heart diseases, cancer, rheumatoid arthritis, asthma, reperfusion injury, inflammatory or autoimmune disorders, transplant rejection, myocardial ischemia, stroke, psoriasis, Kawasaki disease, HIV and AIDS, and septic shock.
20
19. A nucleoside selected from the group consisting of 5'-C-alkylnucleoside, 2'-deoxy-2'-alkylnucleoside, nucleoside 5'-deoxy-5'-dihalo-methylphosphonate, nucleoside 5'-deoxy-5'-difluoro-methylphosphonate, nucleoside 3'-deoxy-3'-dihalo-methylphosphonate, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
25
20. A nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
30

21. A nucleotide triphosphate comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
5
22. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in a talo configuration.
23. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in an allo configuration.
- 10 24. An oligonucleotide comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
- 15 25. An oligonucleotide comprising a moiety having the formula:

wherein B is a nucleotide base or hydrogen; R1, R2 and R3 independently is selected from the group consisting of hydrogen, an alkyl group containing between 2 and 10 carbon atoms inclusive, an amine, an amino acid, and a peptide containing between 2 and 5 amino acids inclusive; and the zigzag lines are independently hydrogen or a bond.

20 26. An oligonucleotide comprising a 3'-amido or peptido group.
27. An oligonucleotide comprising a 5'-amido or peptido group.
28. The oligonucleotide of claim 24, 25, 26, or 27 having enzymatic activity.
25
29. Method for producing an enzymatic nucleic acid molecule having activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one nucleotide having an alkyl group at its 5'-position or 2'-position.
30

30. Method for conversion of a protected allo sugar to a protected talo sugar, comprising the step of contacting said protected allo sugar with triphenyl phosphine, diethylazodicarboxylate, p-nitrobenzoic acid under inversion causing conditions to provide said protected talo sugar.
- 5
31. Method for the synthesis of a nucleoside 5' or a 3'-dihalo-methylphosphonate comprising the step of condensing a difluoromethylphosphonate-containing sugar with a pyrimidine or purine under conditions suitable for forming a nucleoside 5'- or 3'-difluoromethylphosphonate.
- 10
32. The oligonucleotide of claim 3, wherein the normal hammerhead U4 and/or U7 positions are substituted with 2'-NH-amino acid.
33. A method for the synthesis of RNA comprising the step of providing 5-S-alkyltetrazole at a delivered 0.1-1.0 M concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.
- 15
34. A method for the synthesis of RNA comprising the step of providing 5-S-alkyltetrazole at 0.15-0.35 M effective, or final, concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.
- 20
35. A method for the deprotection of RNA comprising the step of providing alkylamine (MA) or NH₄OH/alkylamine (AMA) at between 60°C - 70°C for 5 to 15 minutes to remove any exocyclic amino protecting groups from protected RNA; wherein said alkyl is selected from the group consisting of methyl, ethyl, propyl and butyl.
- 25
36. A method for the deprotection of RNA alkylsilyl protecting groups comprising, contacting said groups with anhydrous triethylamine-hydrogen fluoride (aHF-TEA) trimethylamine or disopropylethylamine at between 60 °C-70 °C for 0.25-24 h.
- 30
37. A method for the purification of an RNA molecule by passing said enzymatic RNA molecule over an HPLC column, wherein said HPCC column is an anion exchange chromatography column.

38. Method for one pot deprotection of RNA comprising, contacting a protected base with anhydrous methyl amine at between 60 °C-70 °C for at least 5 min, cooling the resulting mixture and contacting said mixture with TEA-3HF reagents under conditions which remove a protecting group of the 2'-hydroxyl position.
5
39. Method for synthesizing RNA containing a phosphorothioate linkage comprising the step of contacting 6-10 equivalents of 3H-1,2-benzodithiole-3-one 1,1-dioxide (Beaucage reagent) with the growing RNA chain for 5 seconds with a reaction time of at least 10 300 seconds.
40. Method of synthesizing RNA containing a phosphorothioate linkage comprising the step of achieving coupling with 5-S-ethyltetrazole or 5-S-methyltetrazole prior to sulfurization.
41. Method of claims 38, 39 or 40 wherein said RNA is enzymatically active.
15
42. Method for synthesizing 2'-deoxy-2'-amino-nucleoside phosphoramidite, comprising the step of protecting the 2'-amino group with a N-phtaloyl group.
43. The method of claim 42 wherein the said nucleoside lacks a base.
44. Method for synthesis of RNA comprising the step of: protecting the 2'-position of a nucleotide during said synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group.
20
45. Method for covalently linking a SEM group to the 2'-position of a nucleotide, comprising the step of: contacting a nucleoside with an SEM-containing molecule under SEM bonding conditions.
25
46. The method of claim 45, wherein said conditions comprise dibutyltin oxide and tetrabutylammonium fluoride and SEM-Cl.
47. Method for removal of an SEM group from a nucleoside molecule or an oligonucleotide, comprising the step of: contacting said molecule or oligonucleotide with boron trifluoride etherate ($\text{BF}_3 \cdot \text{OEt}_2$) under SEM removing conditions.
30

48. The method of claim 57 wherein said (BF₃•OEt₂) is provided in acetonitrile.
49. One or more vectors comprising
 - a first nucleic acid sequence encoding a first ribozyme having intramolecular or intermolecular cleaving activity, said first ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif;
 - and a second nucleic acid sequence encoding a second ribozyme having intermolecular cleaving activity, said Second ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif and said second nucleic acid being flanked by other nucleic acid sequences encoding RNA which is cleaved by said first ribozyme to release said second ribozyme from RNA encoded by said vector;
- wherein said first and second nucleic acid sequences may be on the same or separate nucleic acid molecules, and said vector encodes mRNA or comprises RNA which lacks secondary structure which reduces release of said second ribozyme by more than 20%.
50. Cell comprising the vector of claim 49.
51. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs.
52. The RNA molecule of claim 51, wherein said molecule is transcribed by a RNA polymerase III based promoter system.
53. The RNA molecule of claim 51, wherein said molecule is transcribed by a type 2 pol III promoter system.
54. The RNA molecule of claim 51, wherein said molecule is a chimeric tRNA.

55. The RNA molecule of claim 53, said RNA having A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases.
56. The RNA molecule of claim 53, wherein said desired RNA molecule is at the 3' end of said B box.
57. The RNA molecule of claim 53, wherein said desired RNA molecule is in between the said A and the B box.
58. The RNA molecule of claim 53, wherein said desired RNA molecule includes said B box.
59. The RNA molecule of claim 51, wherein said desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA.
60. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 12 bases of said 3' region.
- 15 61. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 15 bases of said 3' region.
62. DNA vector encoding the RNA molecule of claim 51
63. The vector of claim 62, wherein said vector is derived from an AAV or adeno virus.
- 20 64. RNA vector encoding the RNA molecule of claim 51.
65. The vector of claim 64, wherein said vector is derived from an alpha virus or retro virus.
66. The vector of claim 62 wherein the portions of the vector encoding said RNA function as a RNA pol III promoter.
- 25 67. Cell comprising the vector of claim 62.
68. Cell comprising the vector of claim 53.
69. Cell comprising the RNA of claim 51.

70. Method to provide a desired RNA molecule in a cell, comprising introducing said molecule into said cell a RNA comprising a desired RNA molecule, having a 5' terminus able to base pair with at least 8 bases of a 3' region of said RNA molecule.
- 5 71. The method of claim 70, wherein said introducing comprises providing a vector encoding said RNA molecule.
72. Hammerhead ribozyme having 2 or 3 base pairs in stem II with an interconnecting loop of 4 or more bases between said base pairs.
- 10 73. Hairpin ribozyme lacking a substrate moiety, comprising at least six bases in helix 2 and able to base-pair with a separate substrate RNA, wherein the said ribozyme comprises one or more bases 3' of helix 3 able to base-pair with the said substrate RNA to form a helix 5 and wherein the said ribozyme can cleave and/or ligate said separate RNA(s) in *trans*.
- 15 74. The ribozyme of claim 73, wherein said ribozyme comprises six bases in helix 2.
75. The ribozyme of claim 73, having the structure of Fig. 3, wherein each N and N' is independently any base and each dash may represent a hydrogen bond, r is 1-20, q is 2-20, o is 0 - 20, n is 1 - 20 4, and m is 1 - 20.
- 20 76. Method for increasing the activity of a hairpin ribozyme by providing one or more bases 3' of helix 3 able to base-pair with a substrate RNA to form a helix 5.
- 25 77. Trans-cleaving Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.
78. Trans-ligating Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.
- 25 79. The ribozyme of claim 73 having the structure of Fig. 73.
80. The ribozyme of claim 73 having the structure of Fig. 74.
- 30 81. A cell including the ribozyme of any of claims 73-80.

82. An expression vector comprising nucleic acid encoding the ribozyme of any of claims 73-80, in a manner which allows expression of that ribozyme within a cell.
83. A cell including an expression vector of claim 82.
- 5 84. Method for altering in vivo the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule, comprising the steps of:
contacting said nucleic acid molecule in vivo with an oligonucleotide or peptide nucleic acid able to form a duplex or triplex molecule with said nucleic acid molecule, wherein formation of said duplex or triplex molecule directly, or after nucleic acid repair in vivo, causes at least one base in said nucleic acid molecule to be chemically modified to functionally alter the nucleotide base sequence of said nucleic acid sequence.
10
- 15 85. The method of claim 84, wherein said oligonucleotide is of a length sufficient to activate dsRNA deaminase in vivo to cause conversion of an adenine base to inosine in an RNA molecule.
- 20 86. The method of claim 84, wherein said oligonucleotide comprises an enzymatic nucleic acid molecule which is active to chemically modify a base.
87. The method of claim 84, wherein said nucleic acid molecule is DNA or RNA.
88. The method of claim 84, wherein said oligonucleotide comprises a chemical mutagen.
- 25 89. The method of claim 88, wherein said mutagen is nitrous acid.
90. The method of claim 84 wherein said oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methylation of cytosine to 5-methylcytosine.
- 30 91. The method of claim 84, wherein an endogenous mammalian editing system is co-opted to cause said chemical modification.

92. Method for introduction of enzymatic nucleic acid into a cell or tissue, comprising the steps of;
- providing a complex of a first nucleic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;
- and contacting said complex with said cell or tissue under conditions in which said enzymatic nucleic acid molecule is produced in said cell or tissue.
93. Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;
- providing a complex of a first nucleic acid molecule encoding said desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said first nucleic acid molecule lacks a promoter region and said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;
- and contacting said complex with said cell or tissue under conditions in which said desired acid molecule is produced in said cell or tissue.
94. Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;
- providing a complex of a first nucleic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired

structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

5 and wherein said second nucleic acid further comprises a localization factor;

and contacting said complex with said cell or tissue under conditions in which said desired nucleic acid molecule is produced in said cell or tissue.

- 10 95. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.
- 15 96. Complex of a first nucleic acid molecule encoding a desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said first nucleic acid molecule lacks a promoter region and said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.
- 20 97. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.
- 25
- 30

first nucleic acid under said conditions, and wherein said second nucleic acid further comprises a localization factor.

1/103

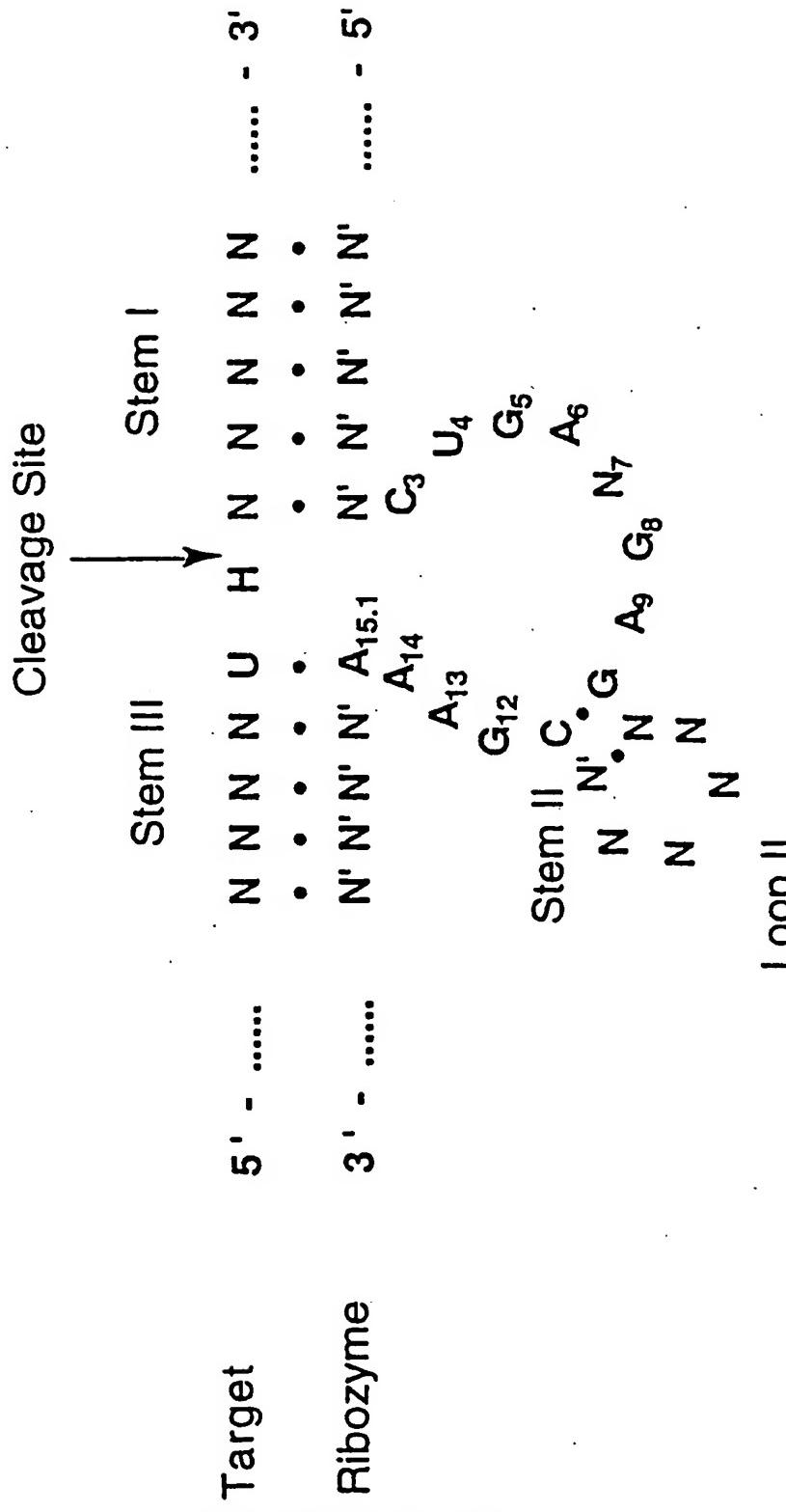
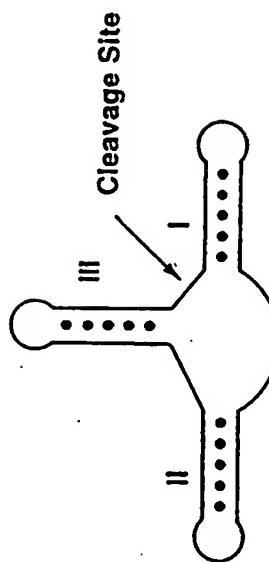


FIG. I.

SUBSTITUTE SHEET (RULE 26)

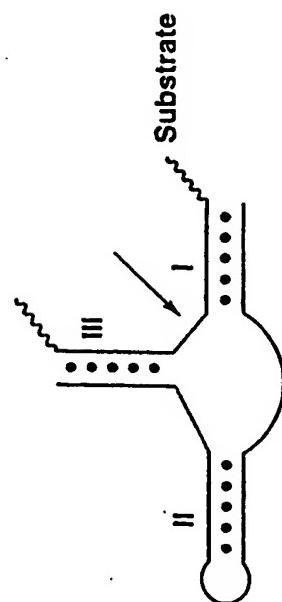
2/103

FIG. 2a.



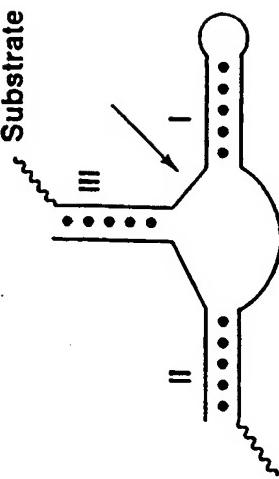
a

FIG. 2c.



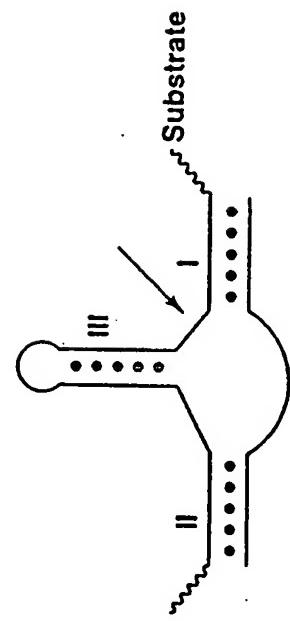
c

FIG. 2d.



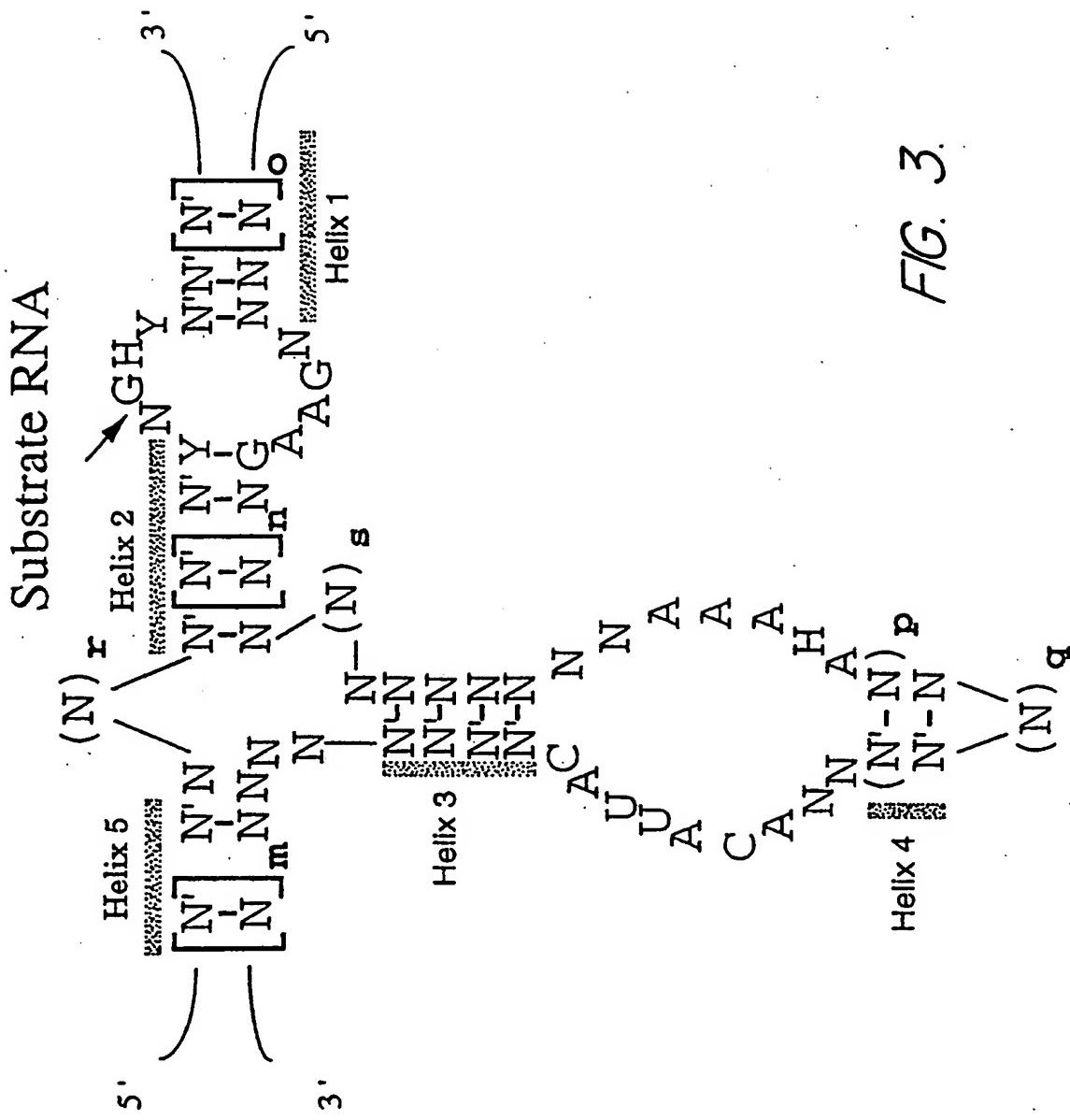
d

FIG. 2b.

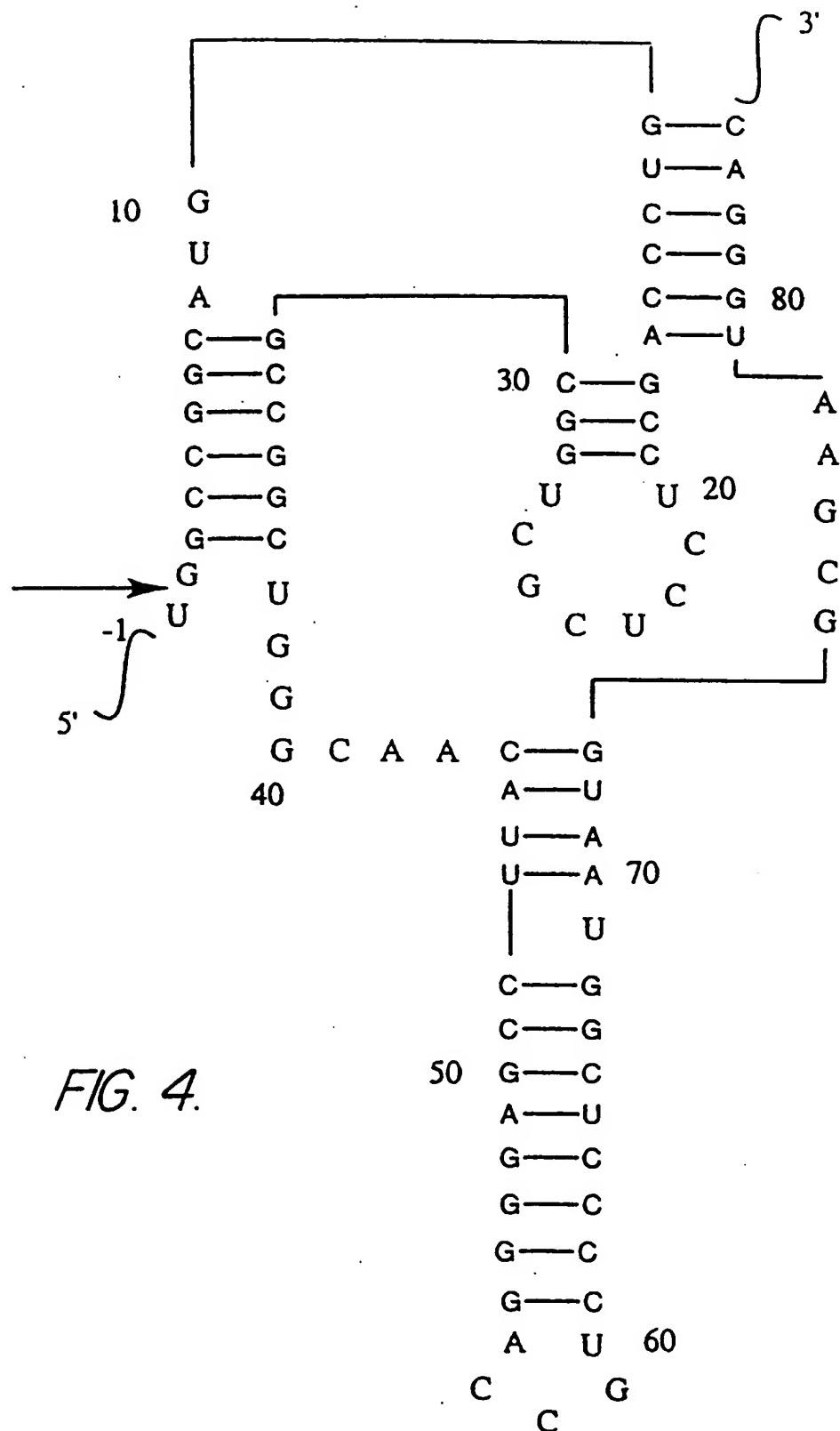


b

3/103

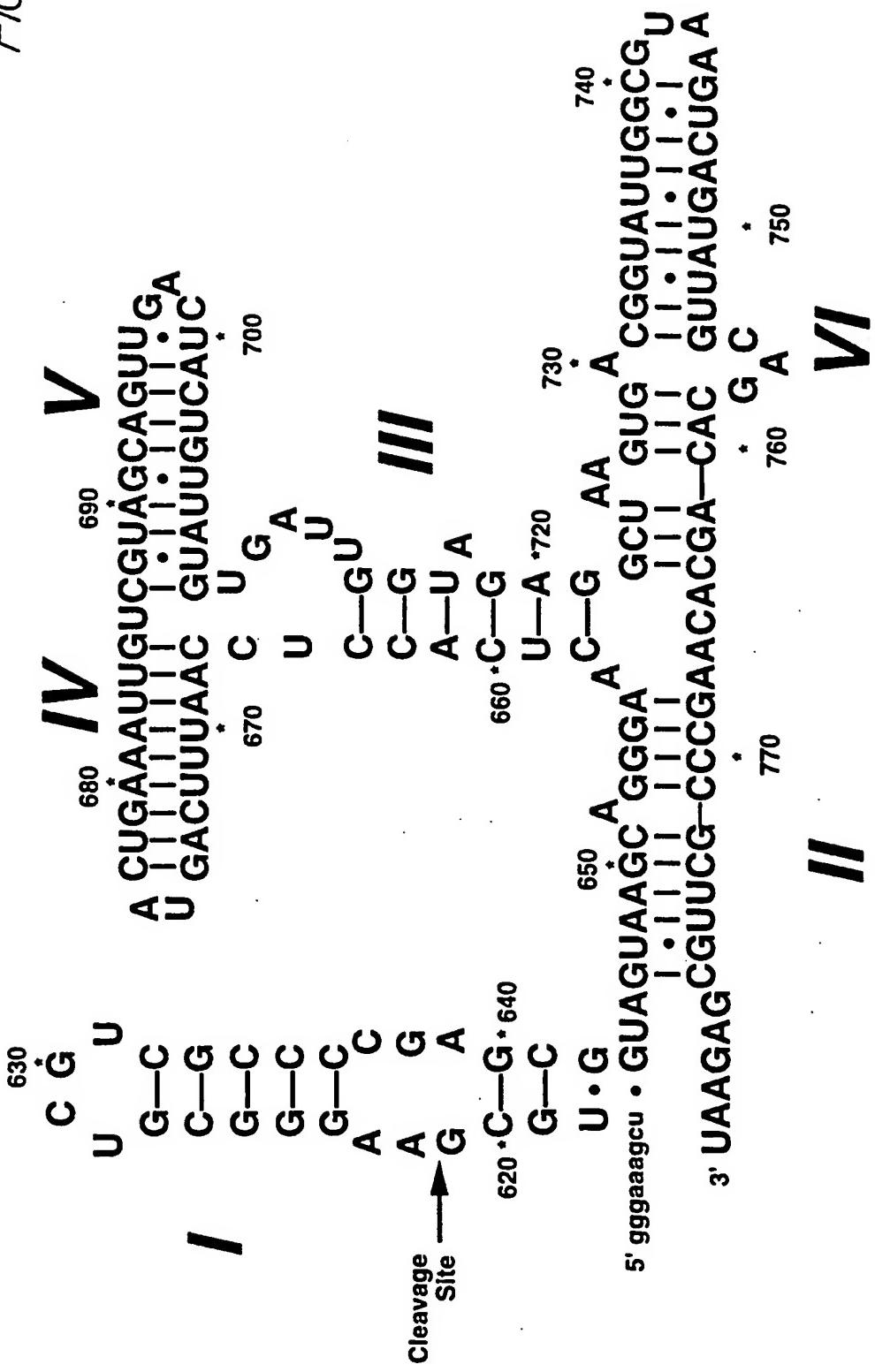


4 / 103



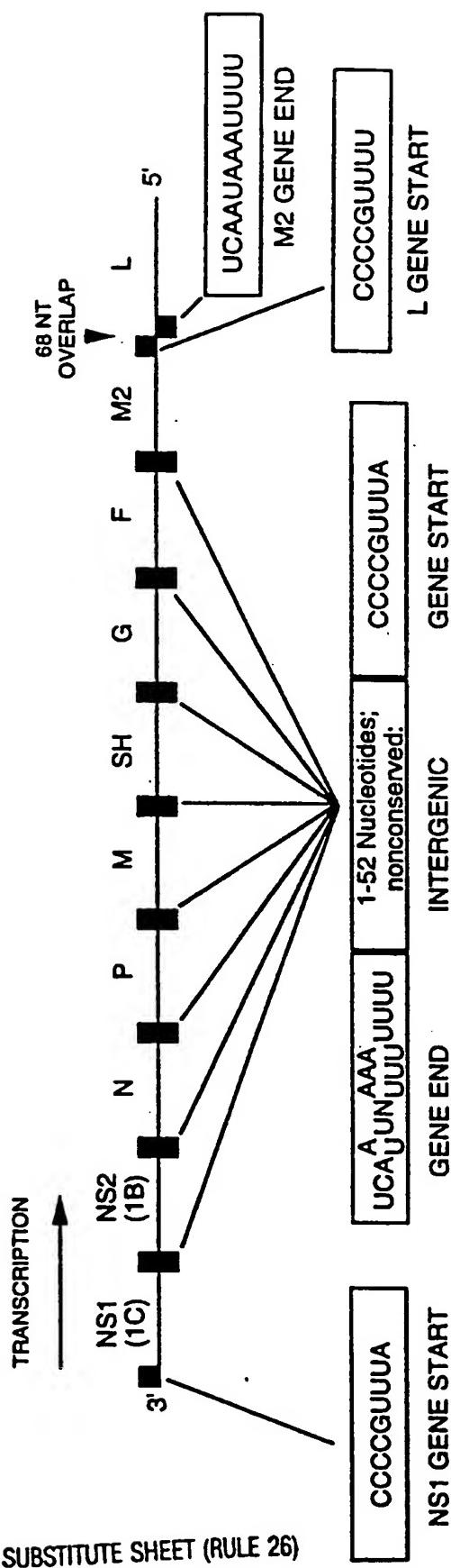
5/103

FIG. 5.



SUBSTITUTE SHEET (RULE 26)

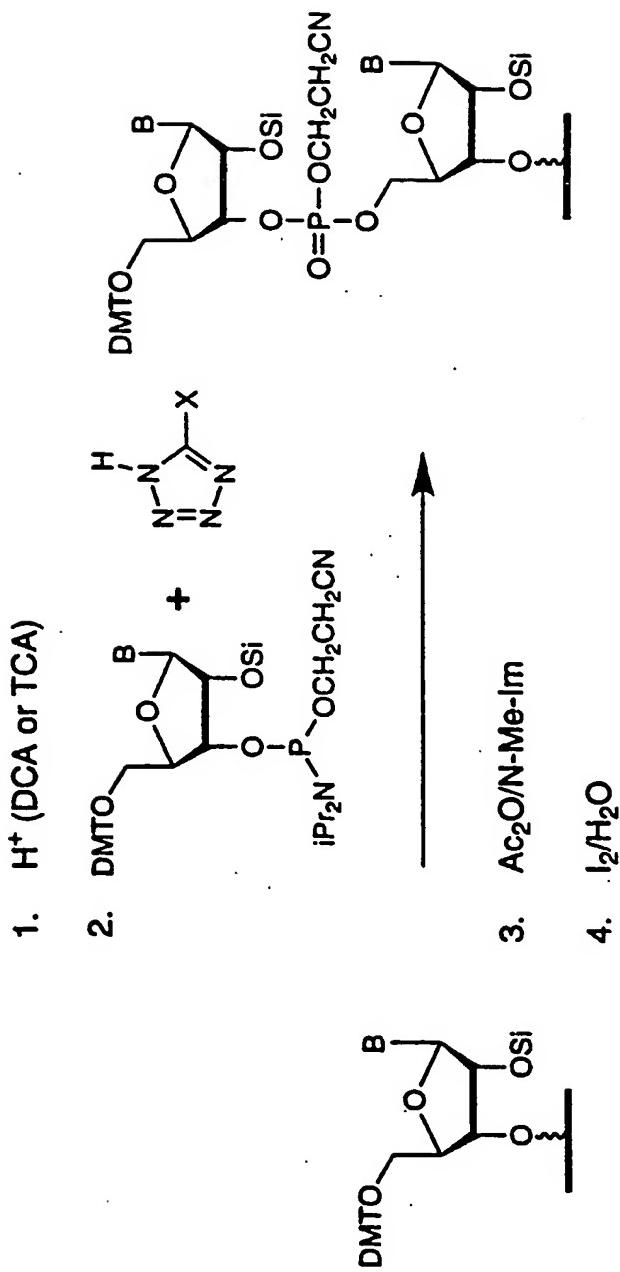
FIG. 6.



Adapted from Virology, Second Edition, Edited by B.N. Fields, 1990.

7/103

FIG. 7.



8/103

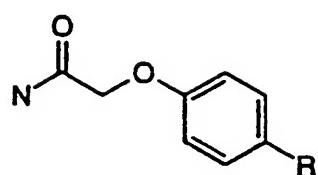
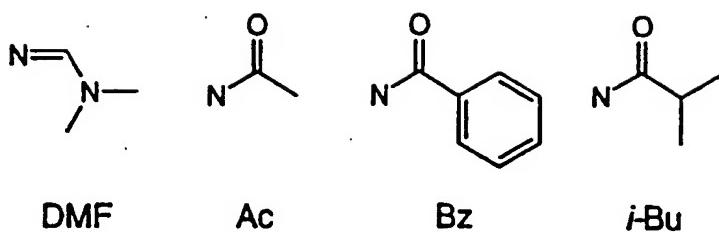
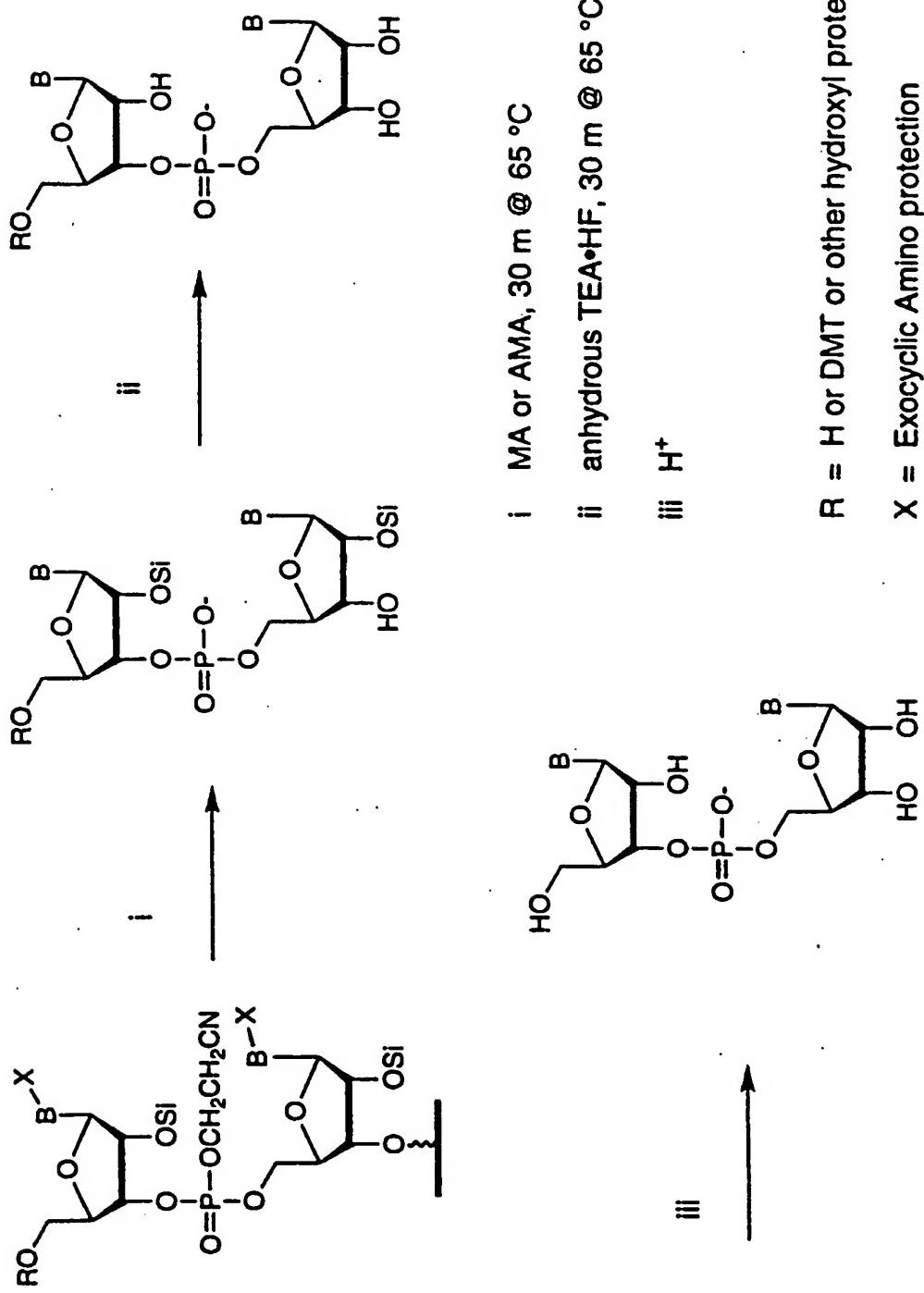


FIG. 8.

- R = H = PAC
R = *t*Bu = TAC
R = *i*Pr = *i*PPAC

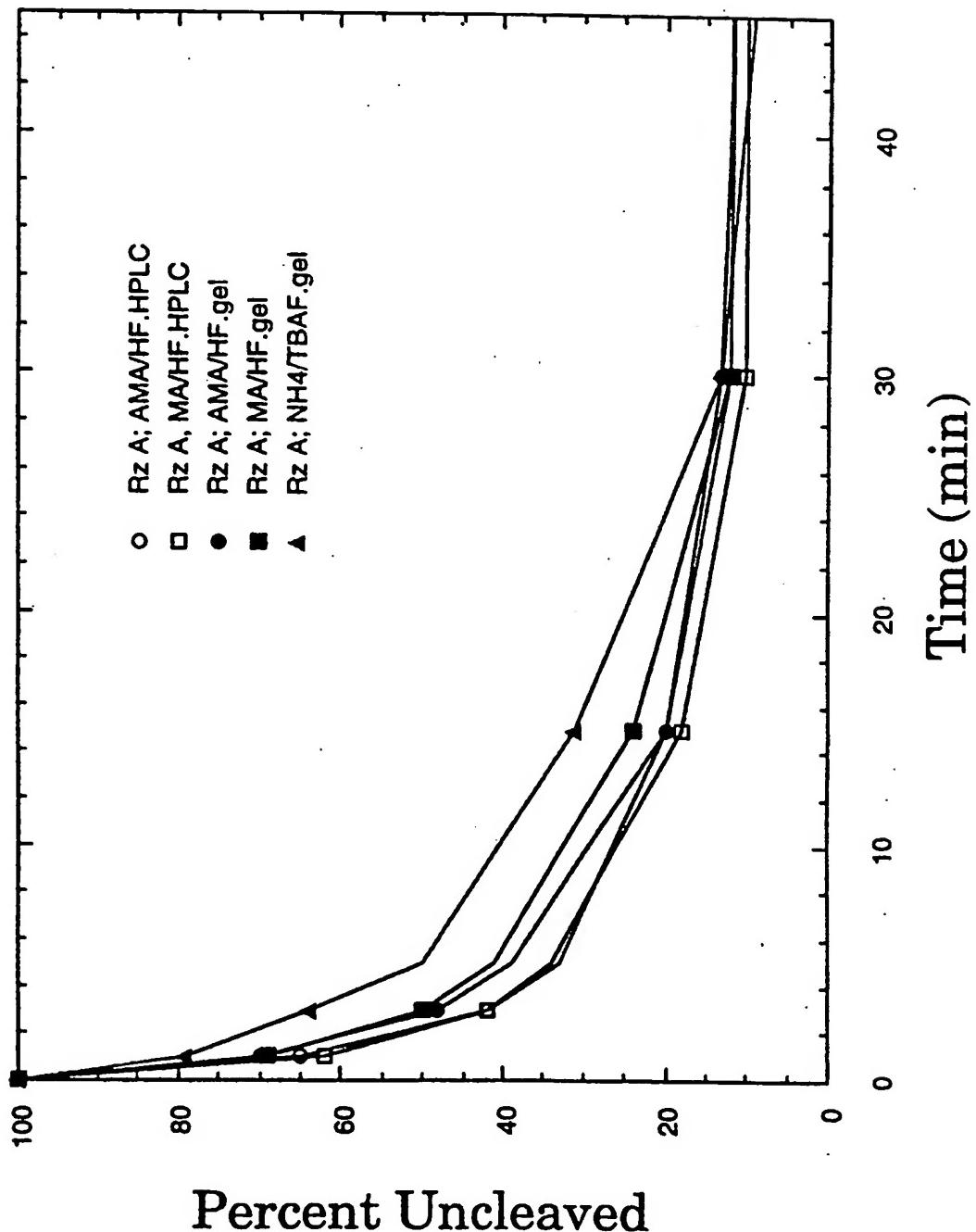
9/103

FIG. 9.



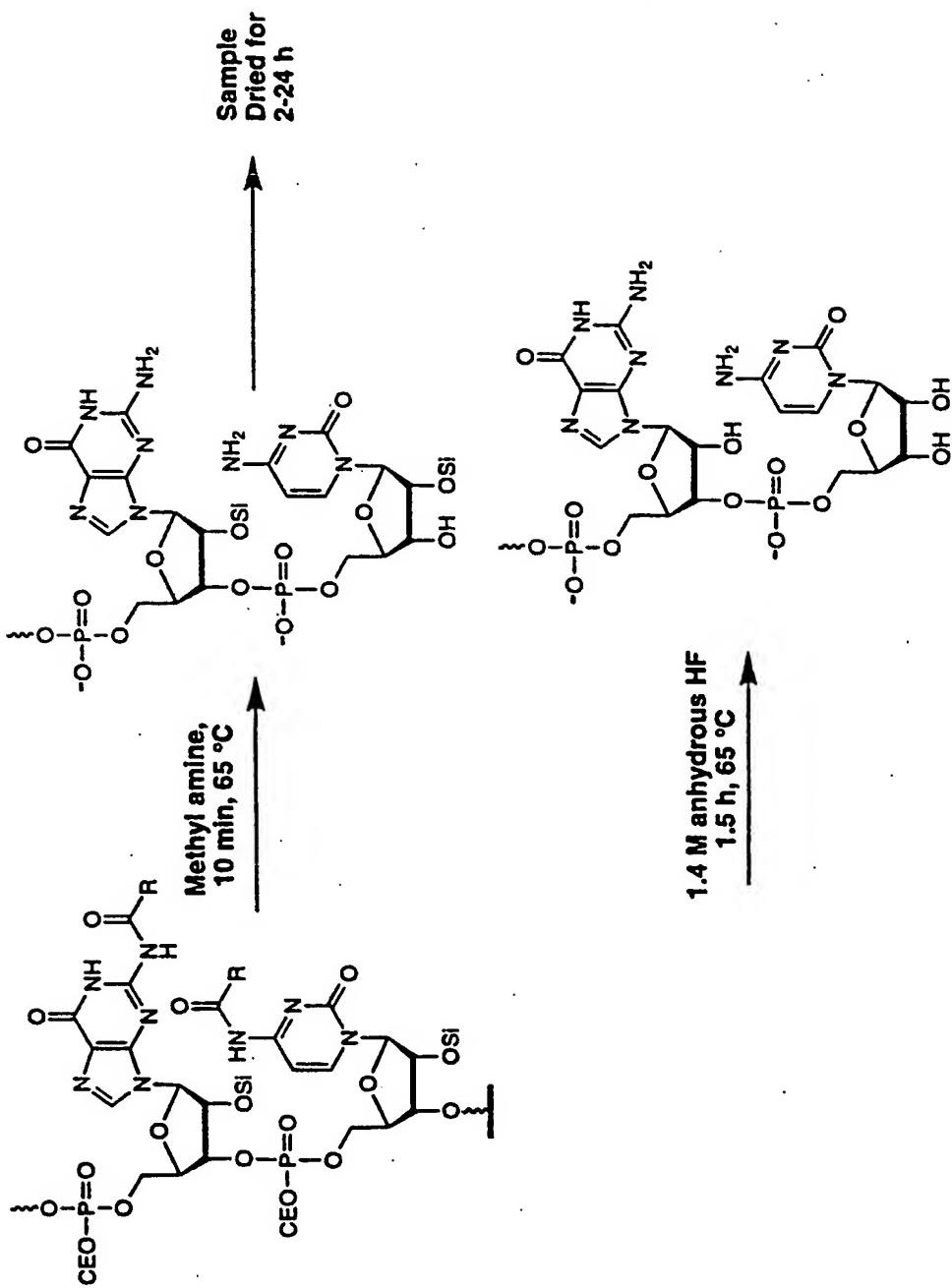
10/103

FIG. 10.

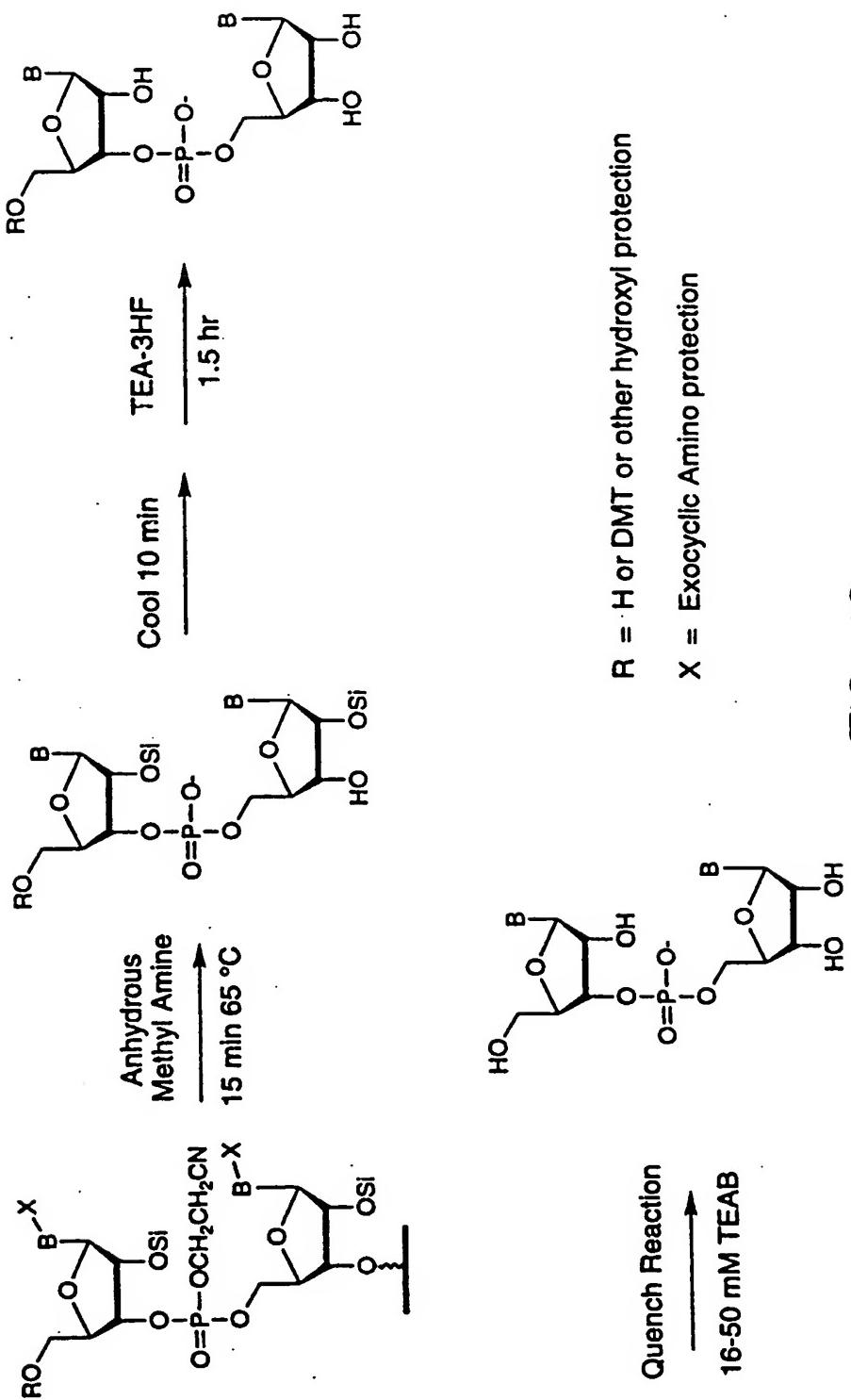


11/103

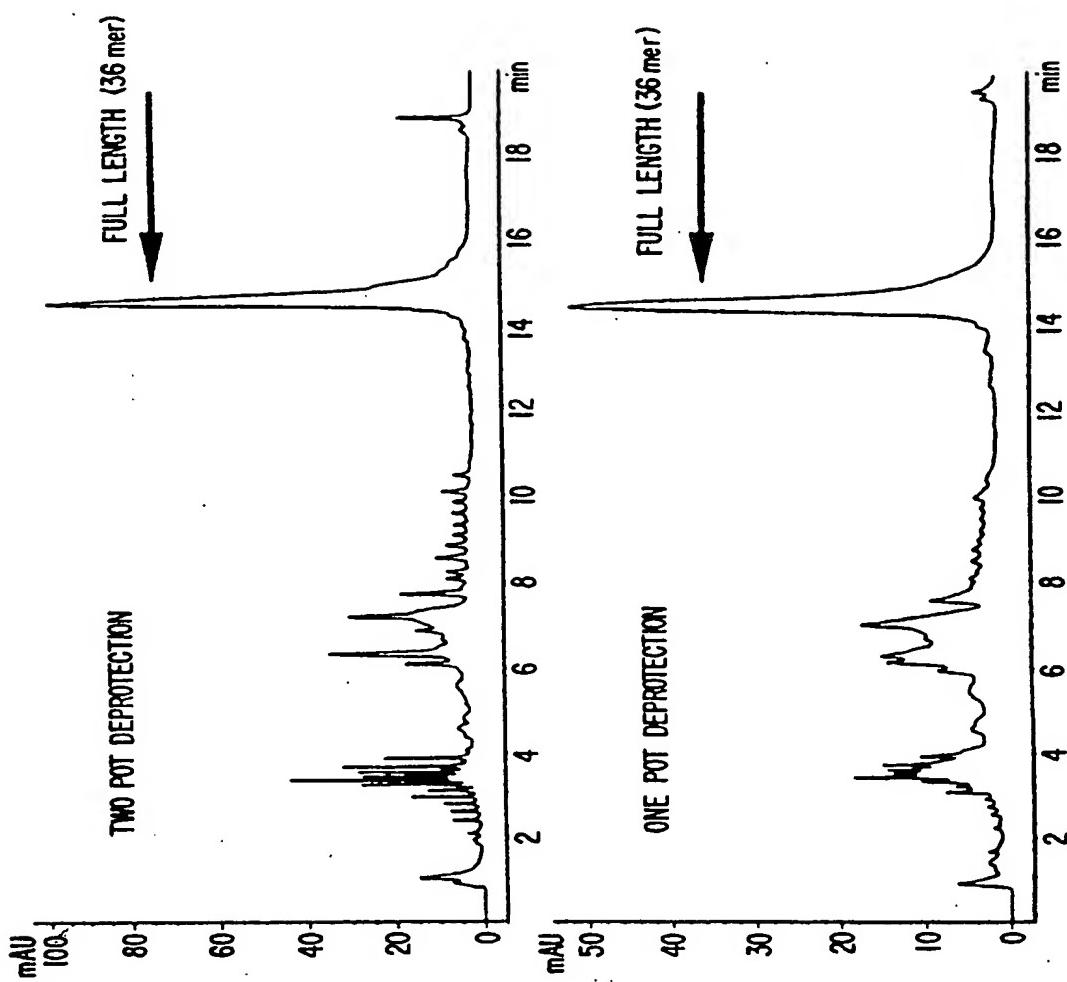
FIG. 1.



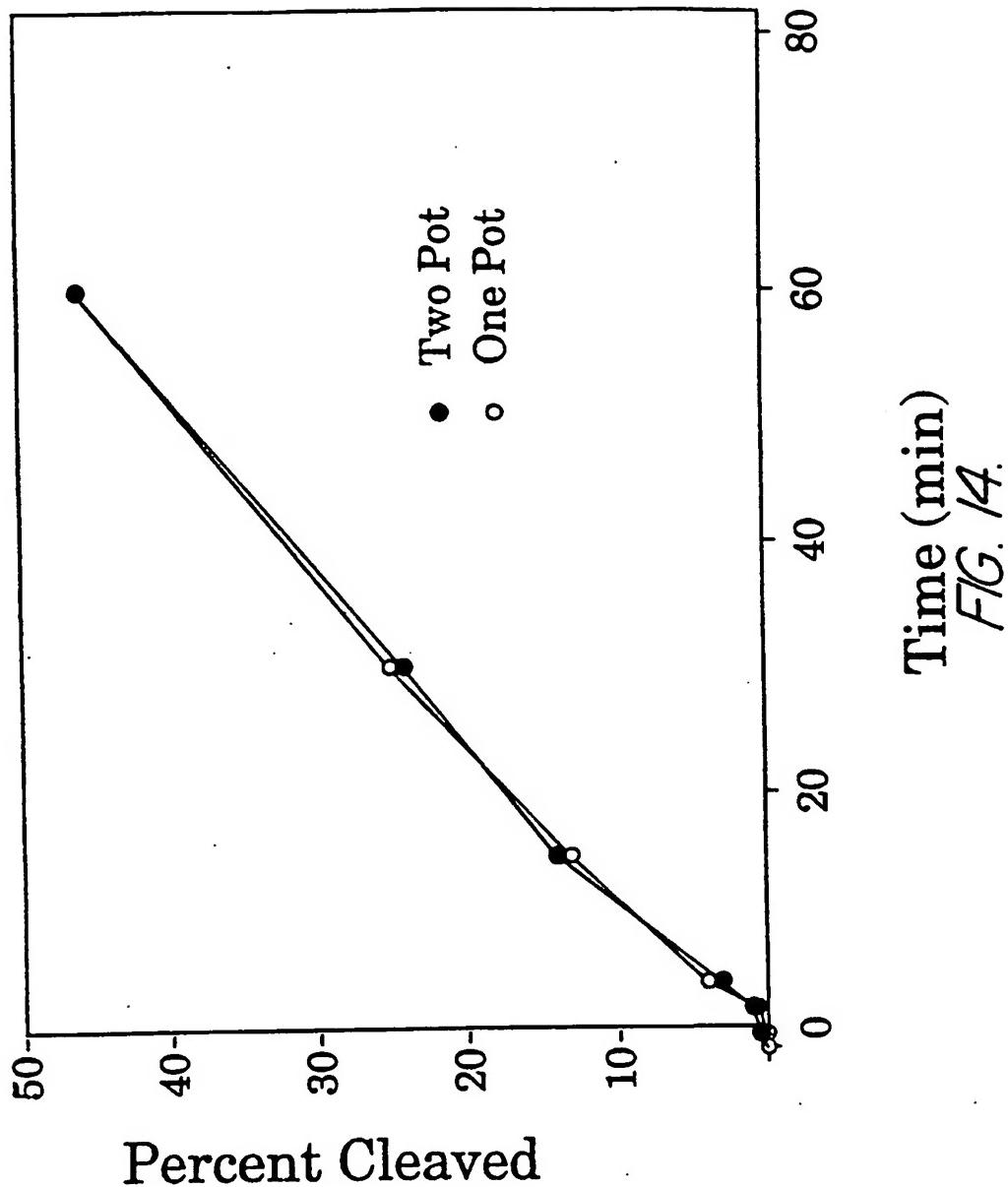
12/103



13/103

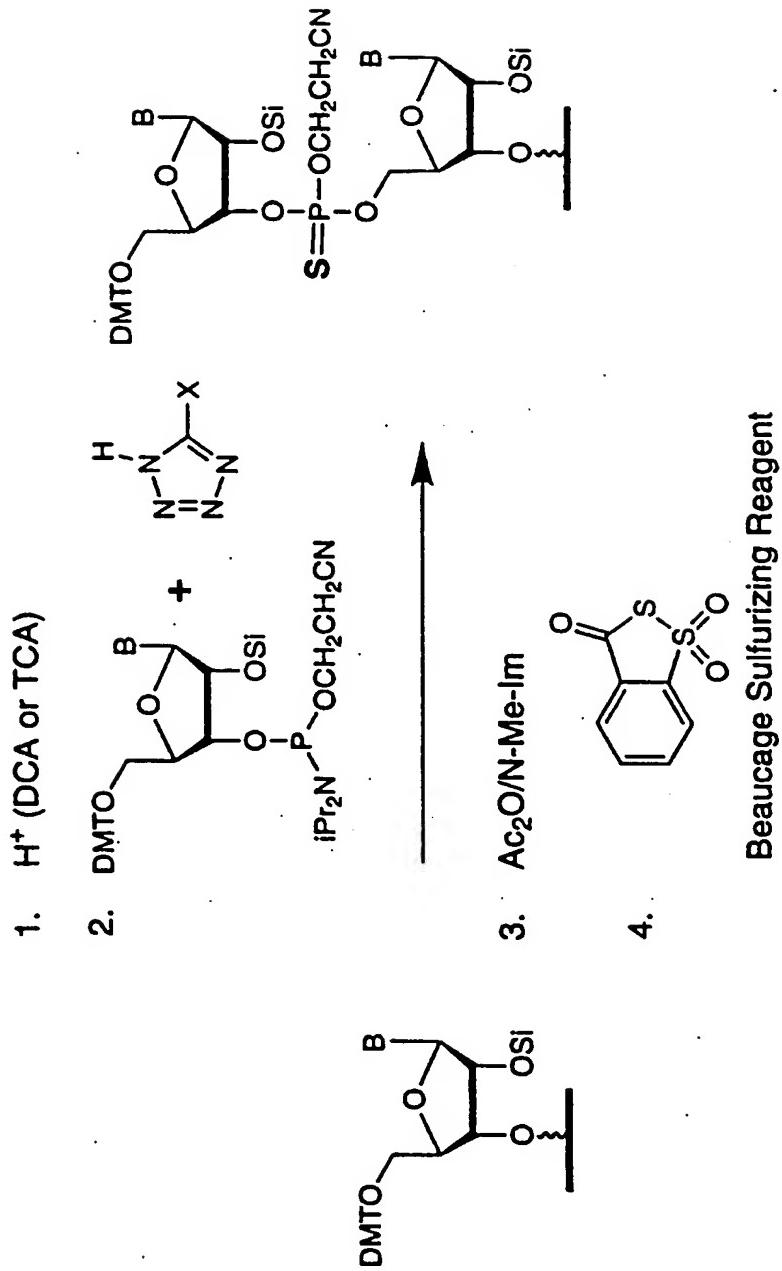


14/103

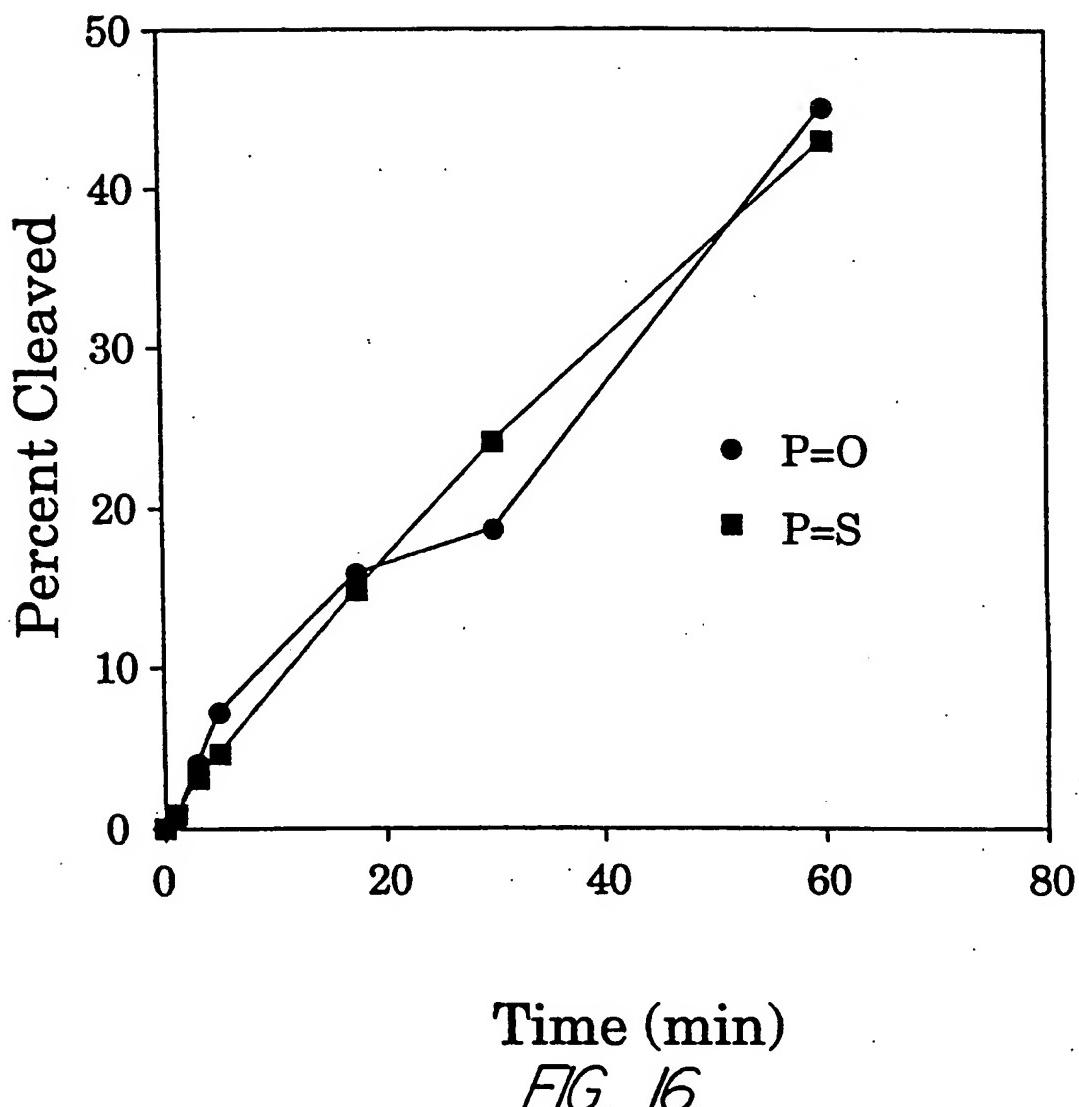


15/103

FIG. 15.



16/103



17/103

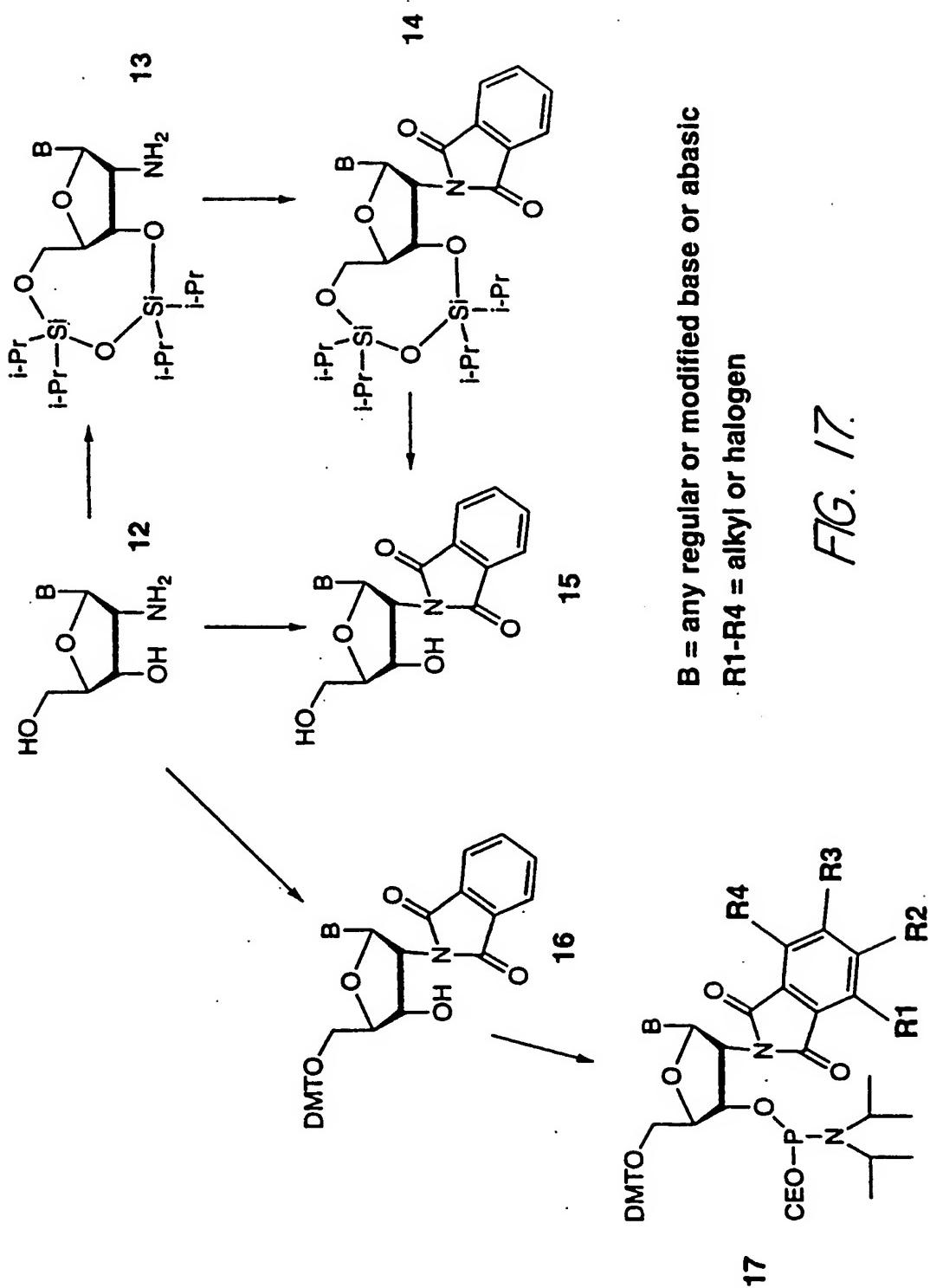
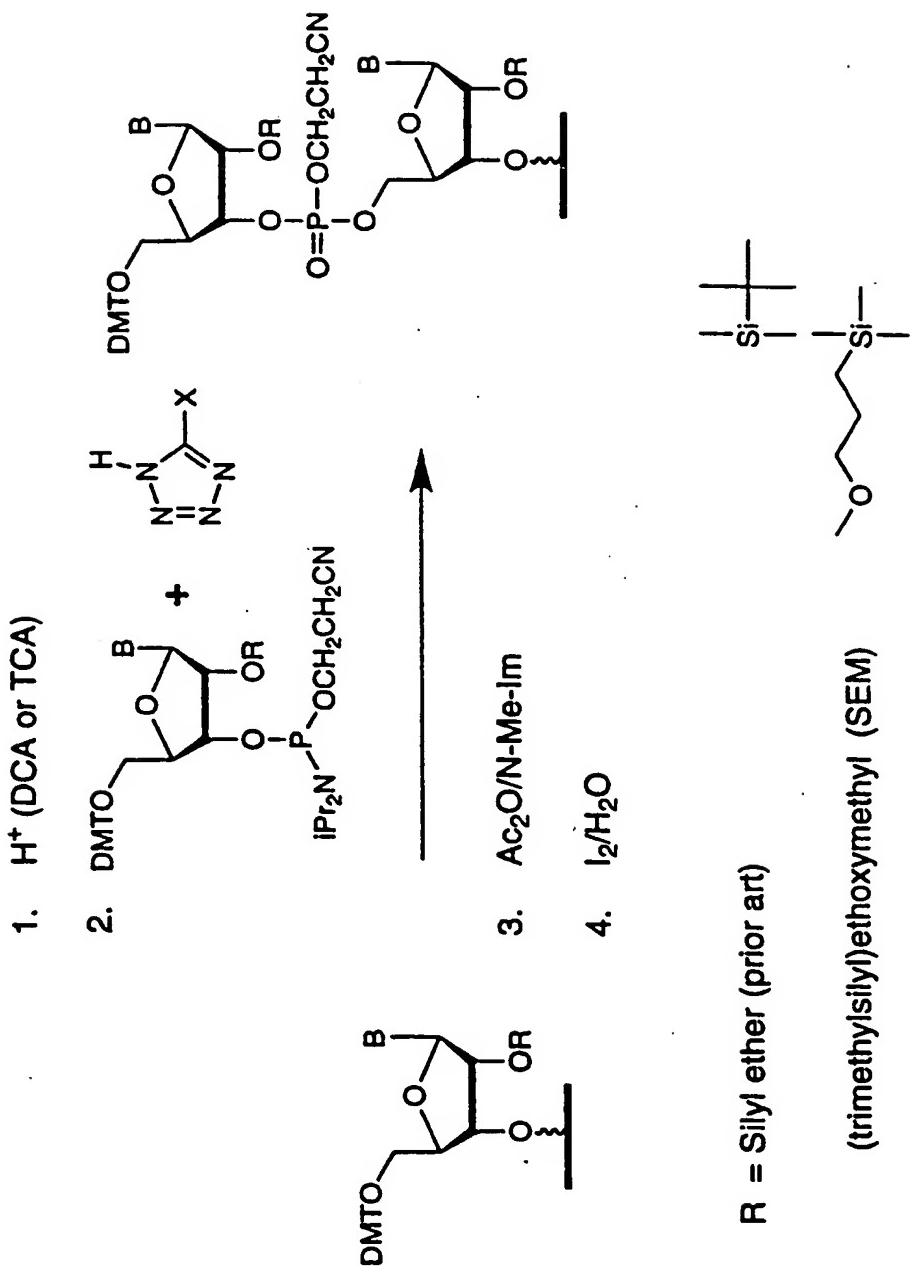


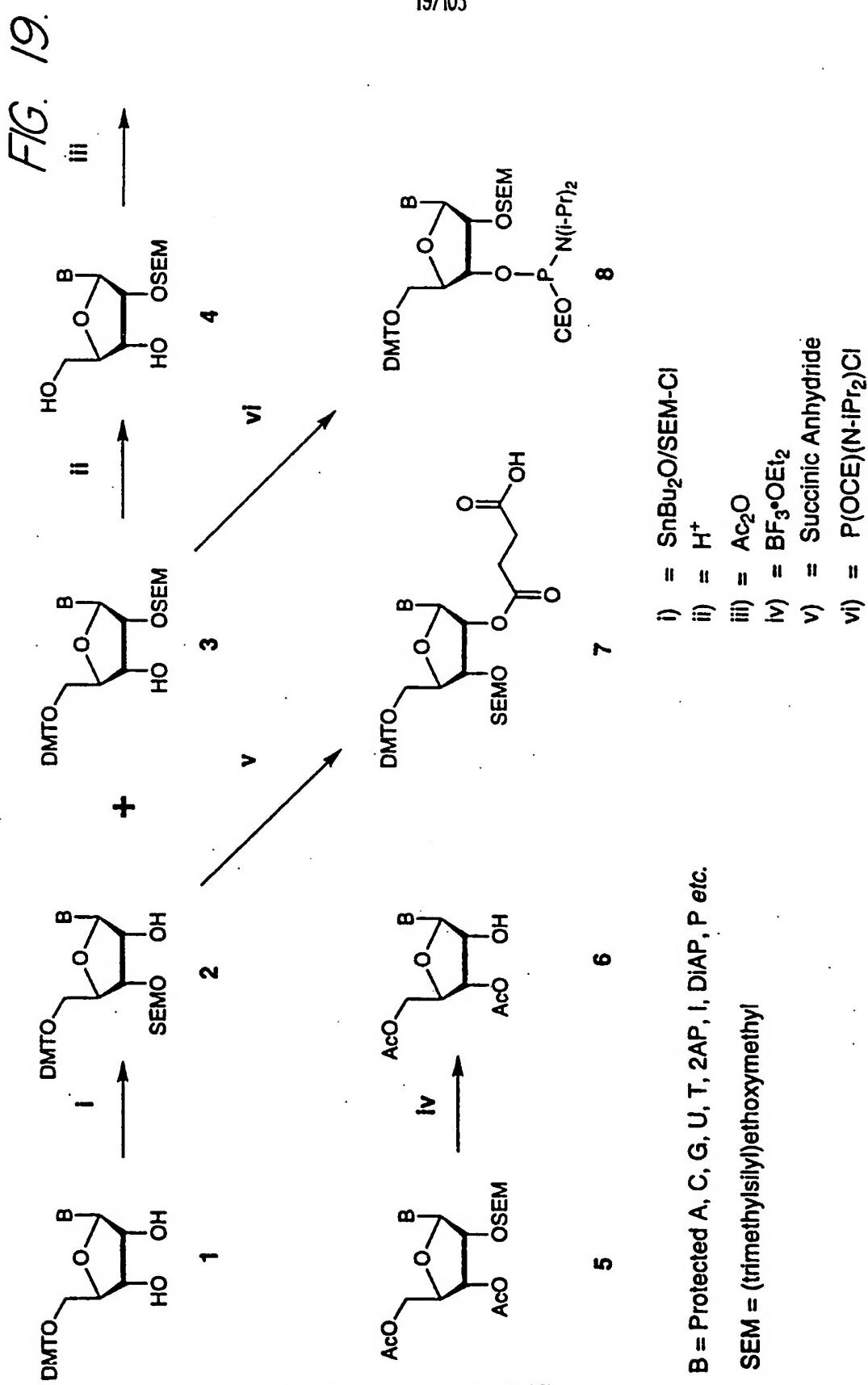
FIG. 17

18/103

FIG. 18.



19/103



SUBSTITUTE SHEET (RULE 26)

20/103

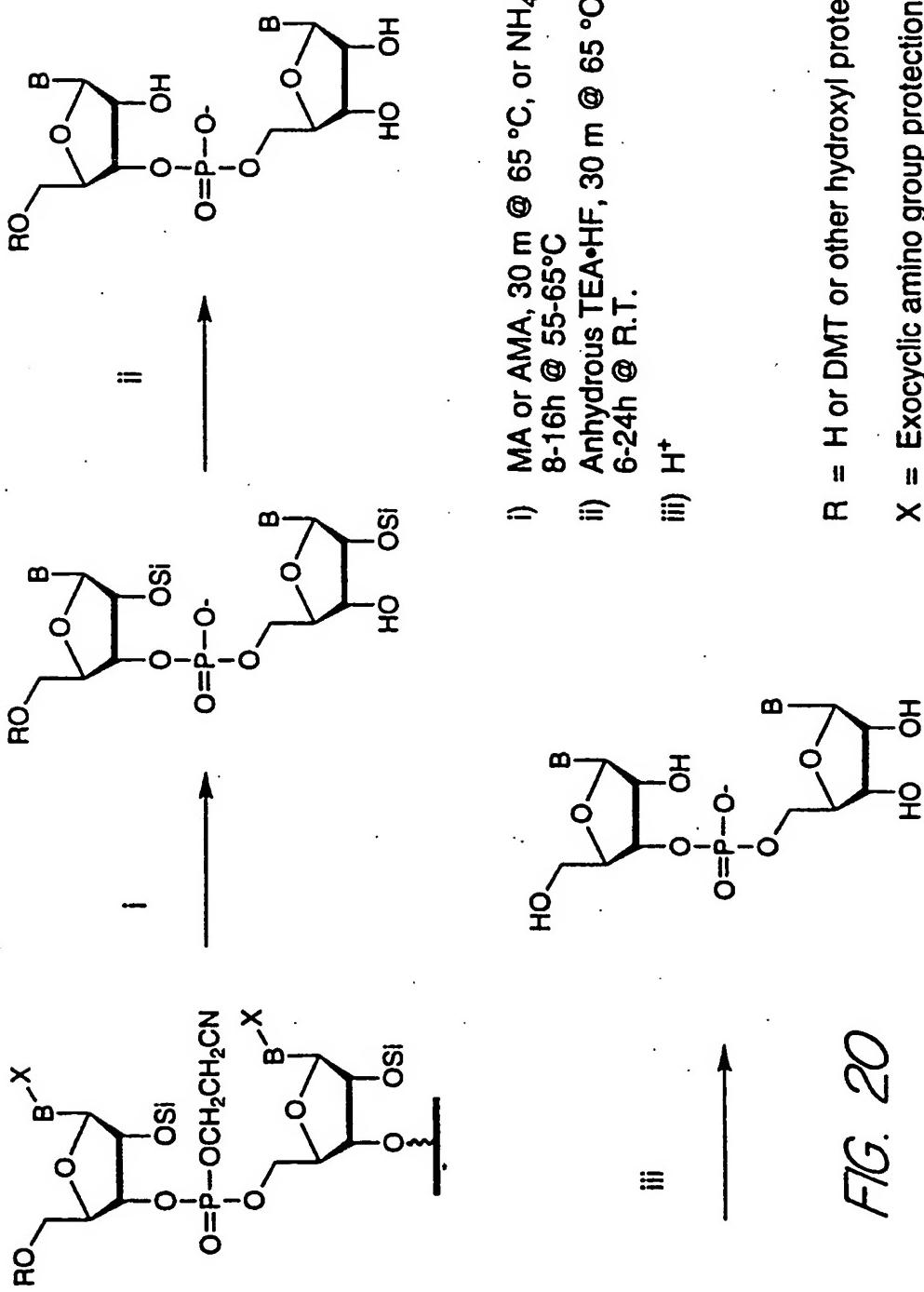
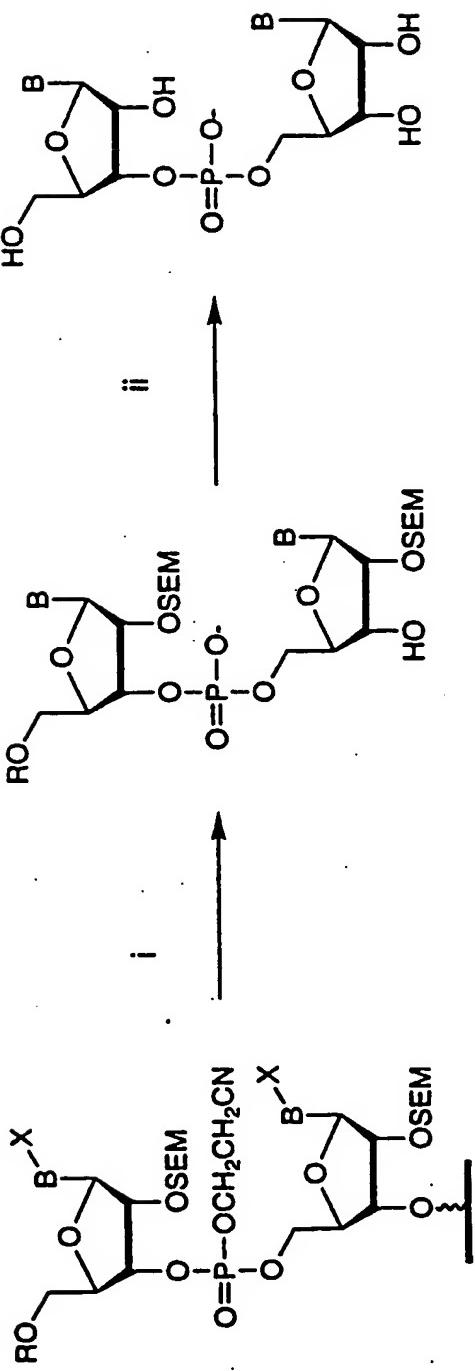


FIG. 20

21/103

FIG. 21.



i) MA or AMA, 30 m @ 65 °C or NH₄OH/EtOH, 8-16h @ 55-65°C

ii) BF₃•OEt₂

SEM = (trimethylsilyl)ethoxymethyl

R = H or DMT or other hydroxyl protection

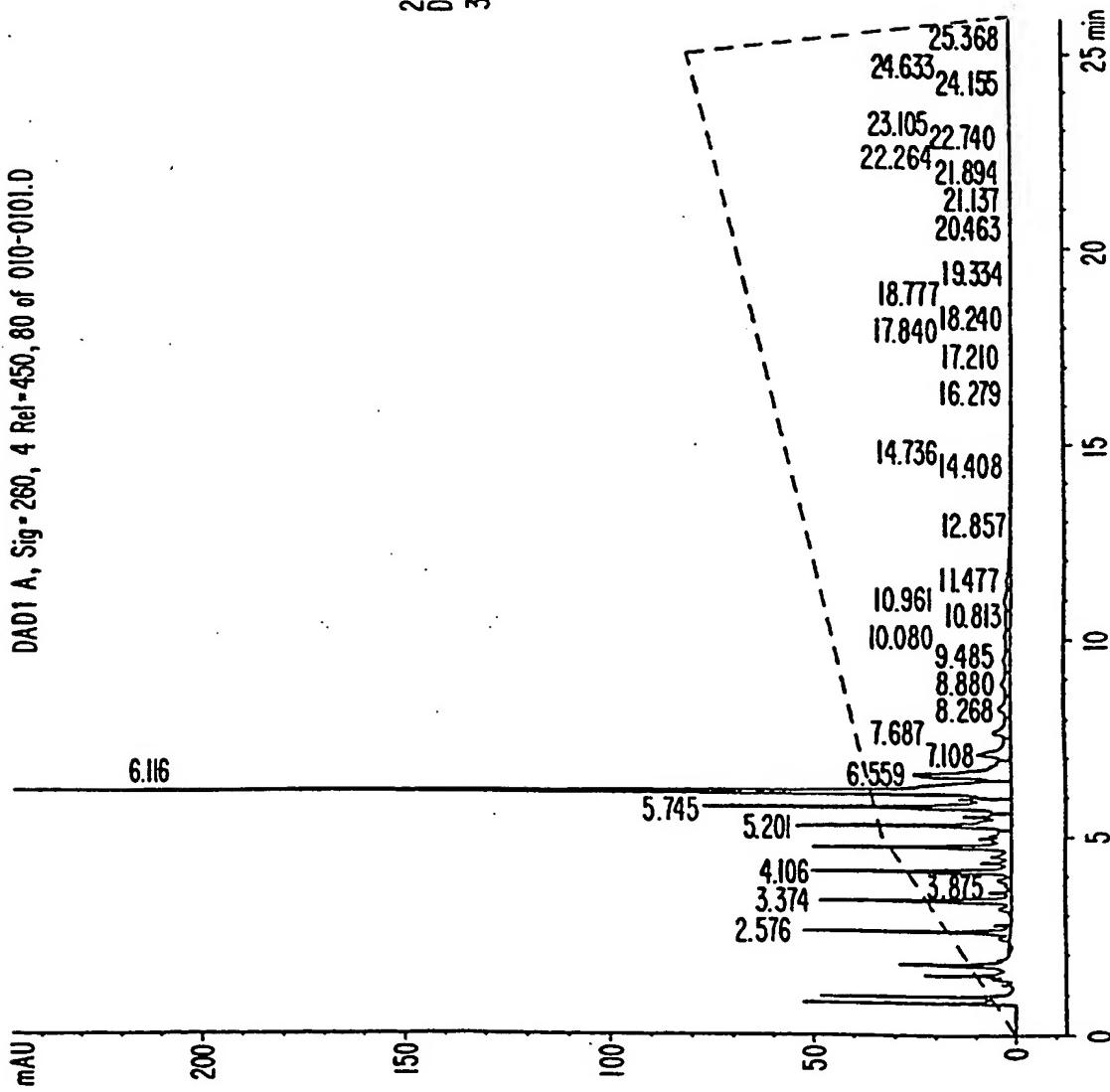
X = Exocyclic amino group protection

22/103

DAD1 A, Sig = 280, 4 Rel = 450, 80 of 010-0101.D

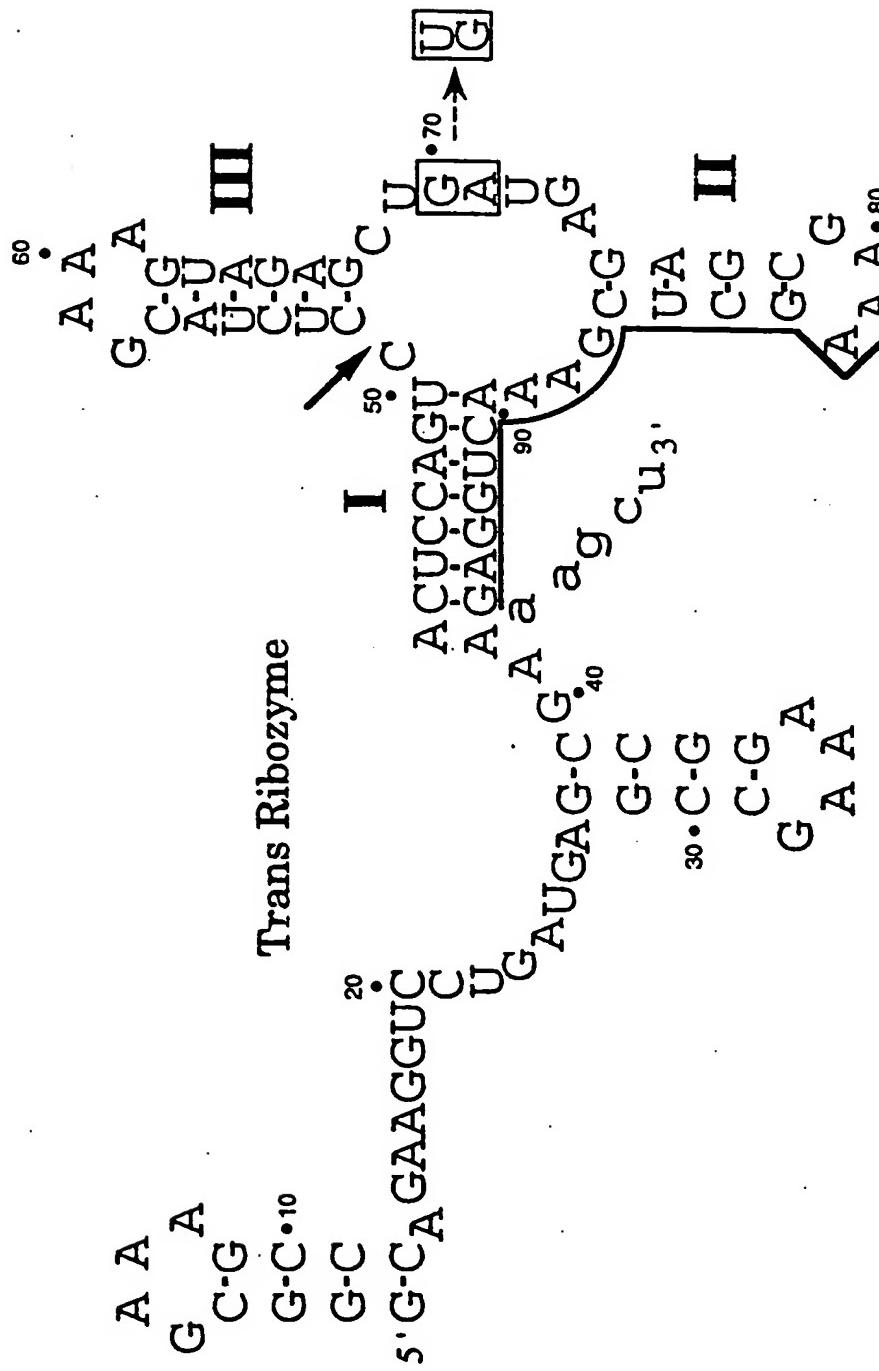
FIG. 22.

2'-O-SEM PROTECTED U 10-mer
DEPROTECTED WITH $\text{BF}_3 \cdot \text{OEt}_2$
30m, 3eq./nucleotide



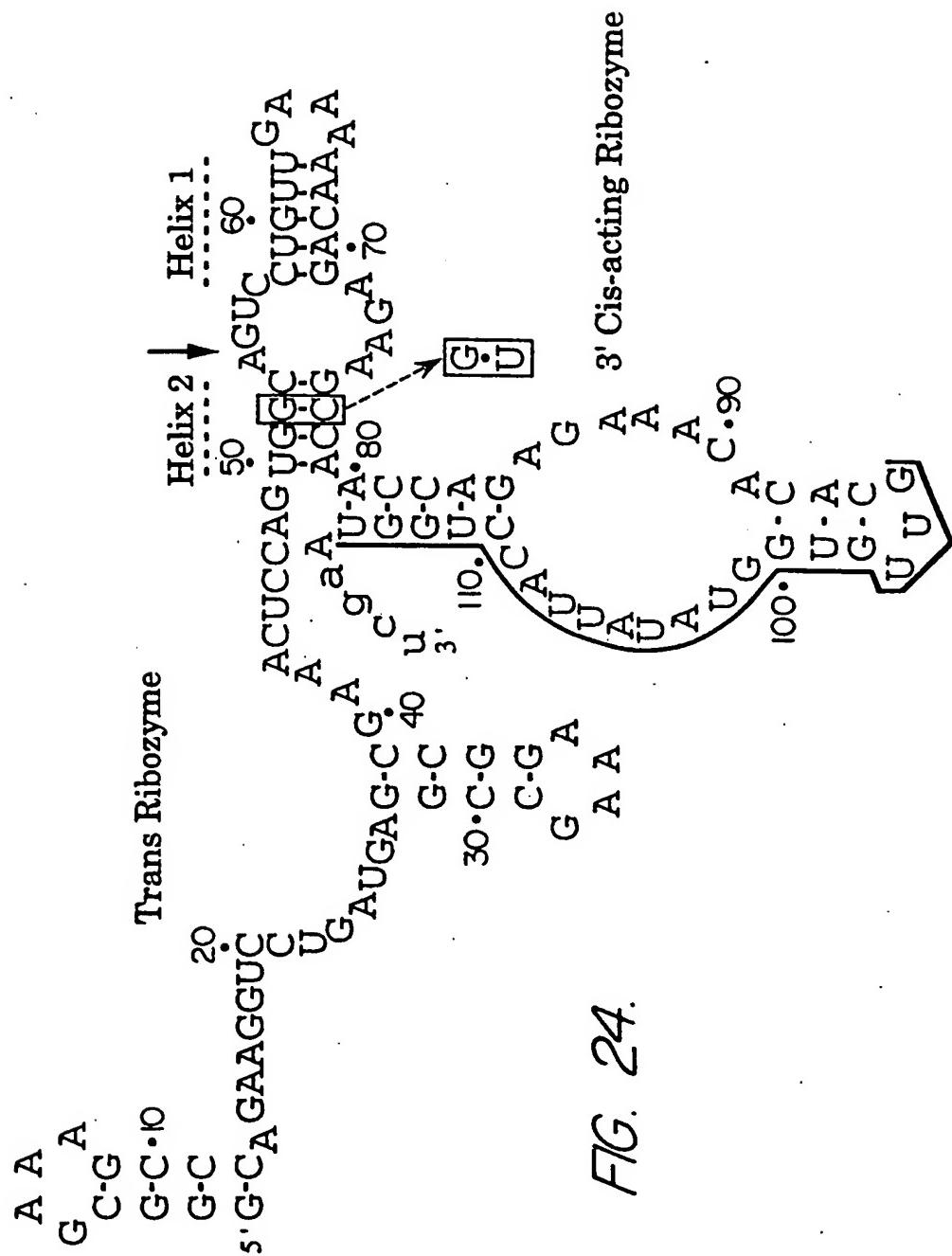
23/103

FIG. 23.



3' Cis-acting Ribozyme

24/103



25/103

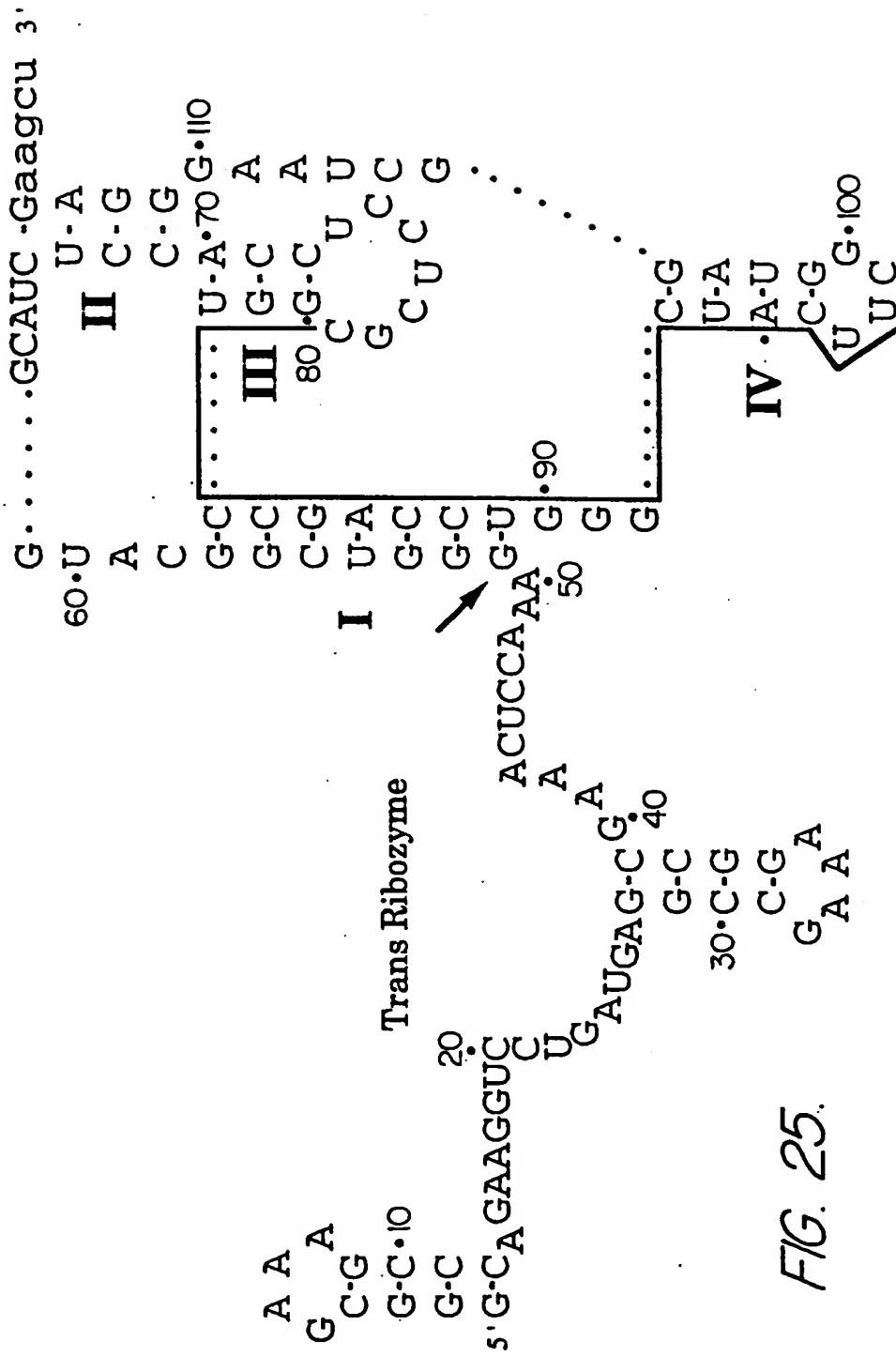
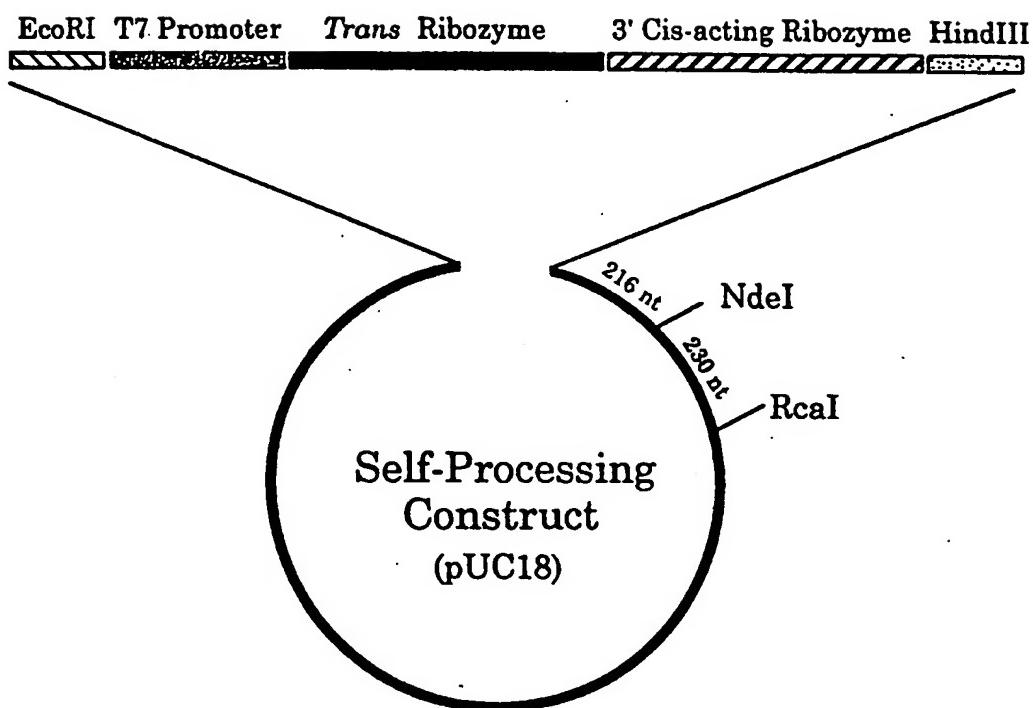


FIG. 25.

3' Cis-acting Ribozyme

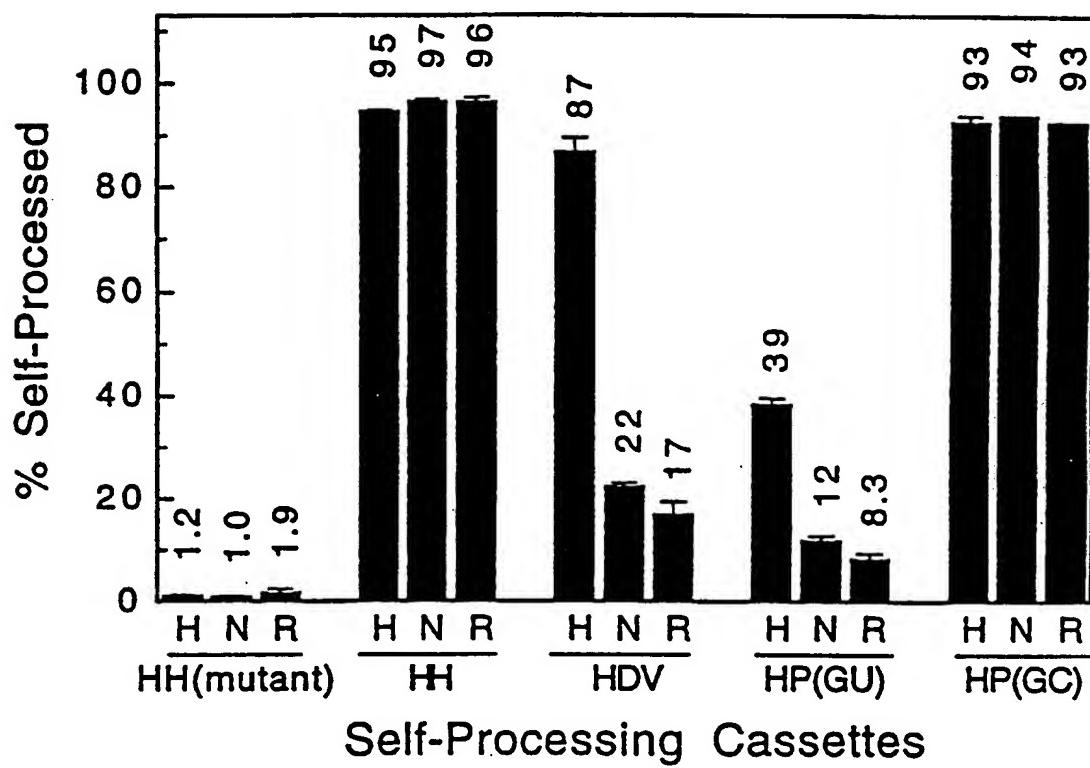
26/103

FIG. 26.



27/103

FIG. 27.



28/103

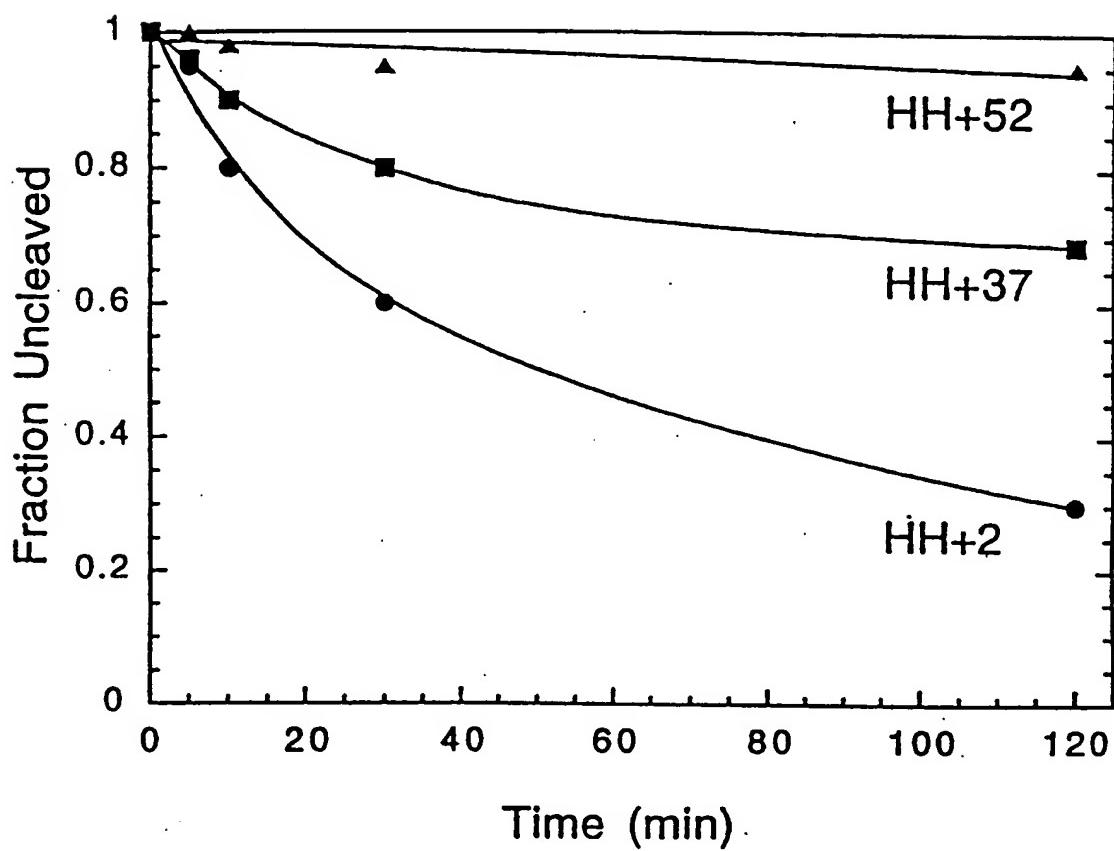
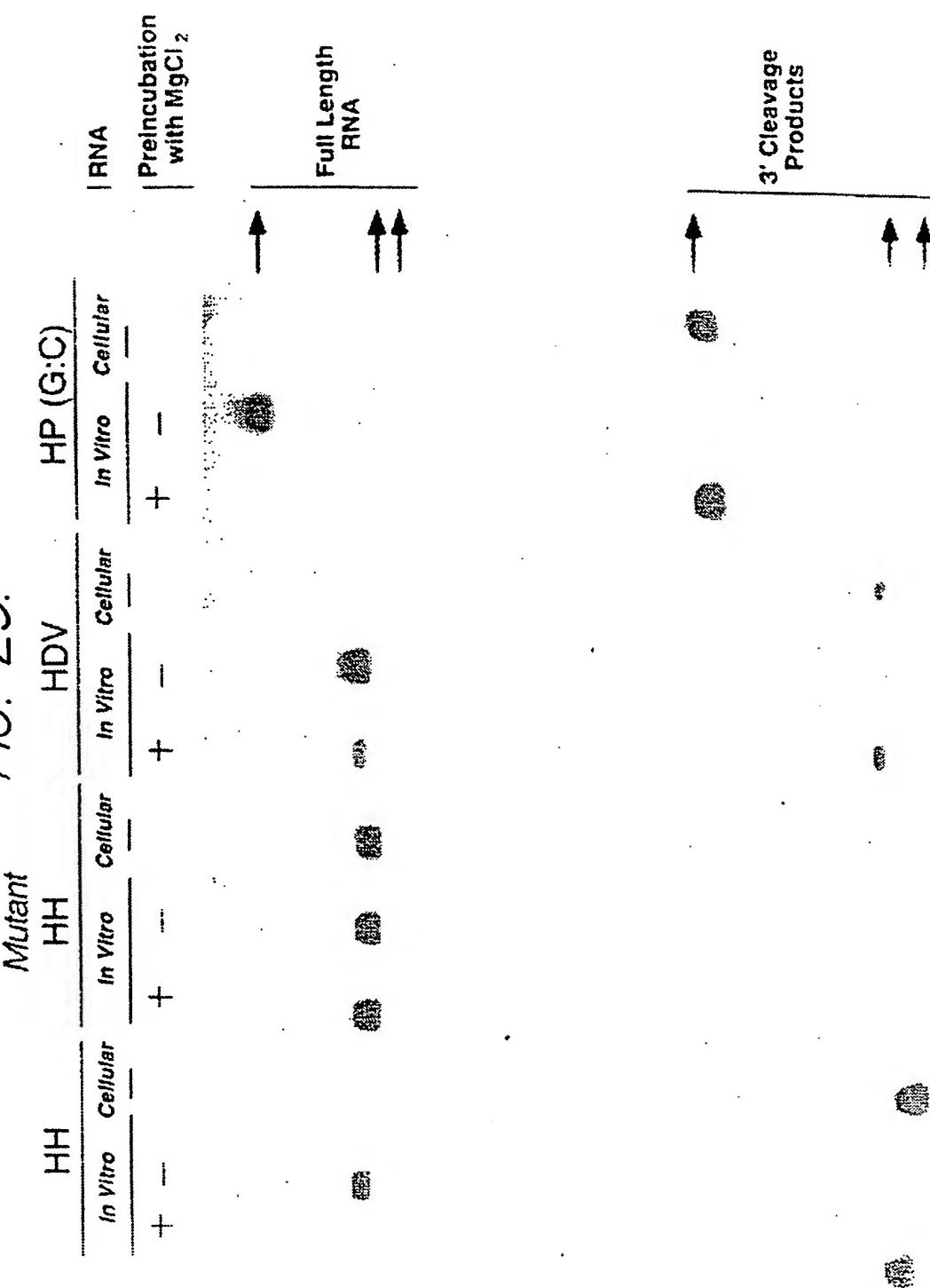


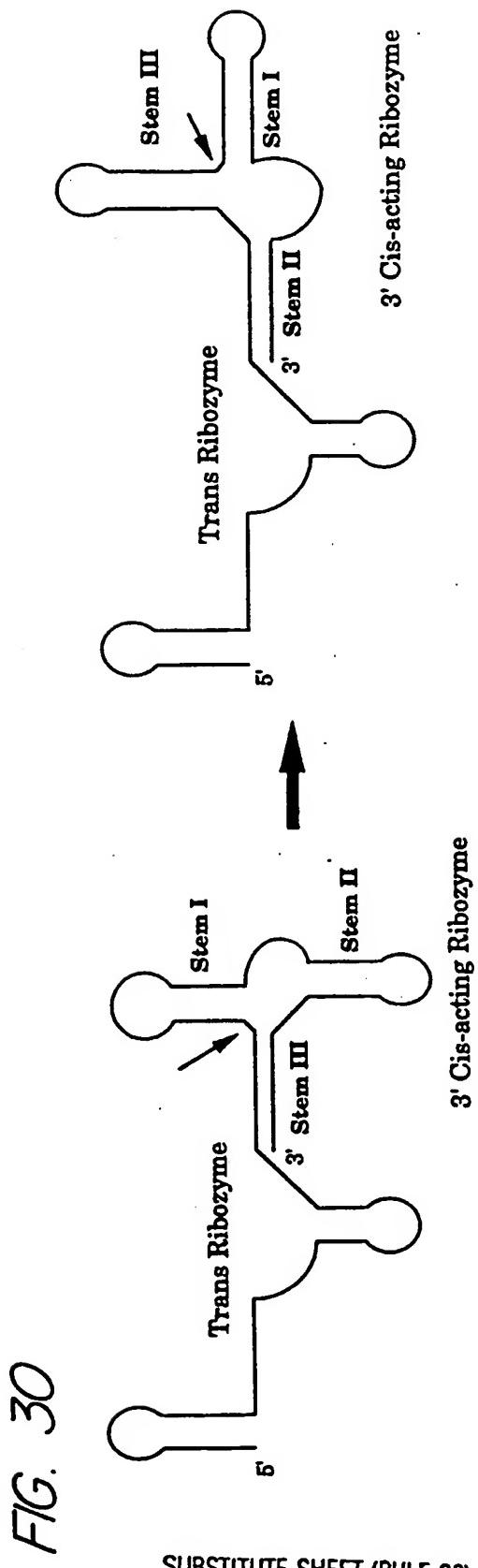
FIG. 28.

29/103

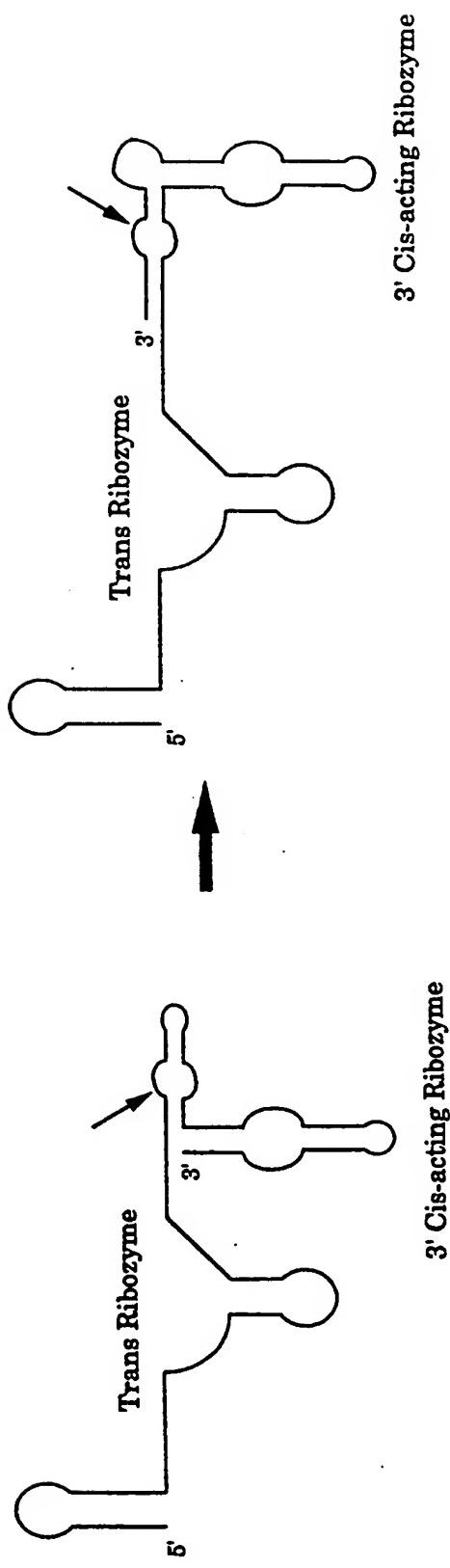
FIG. 29.



30/103

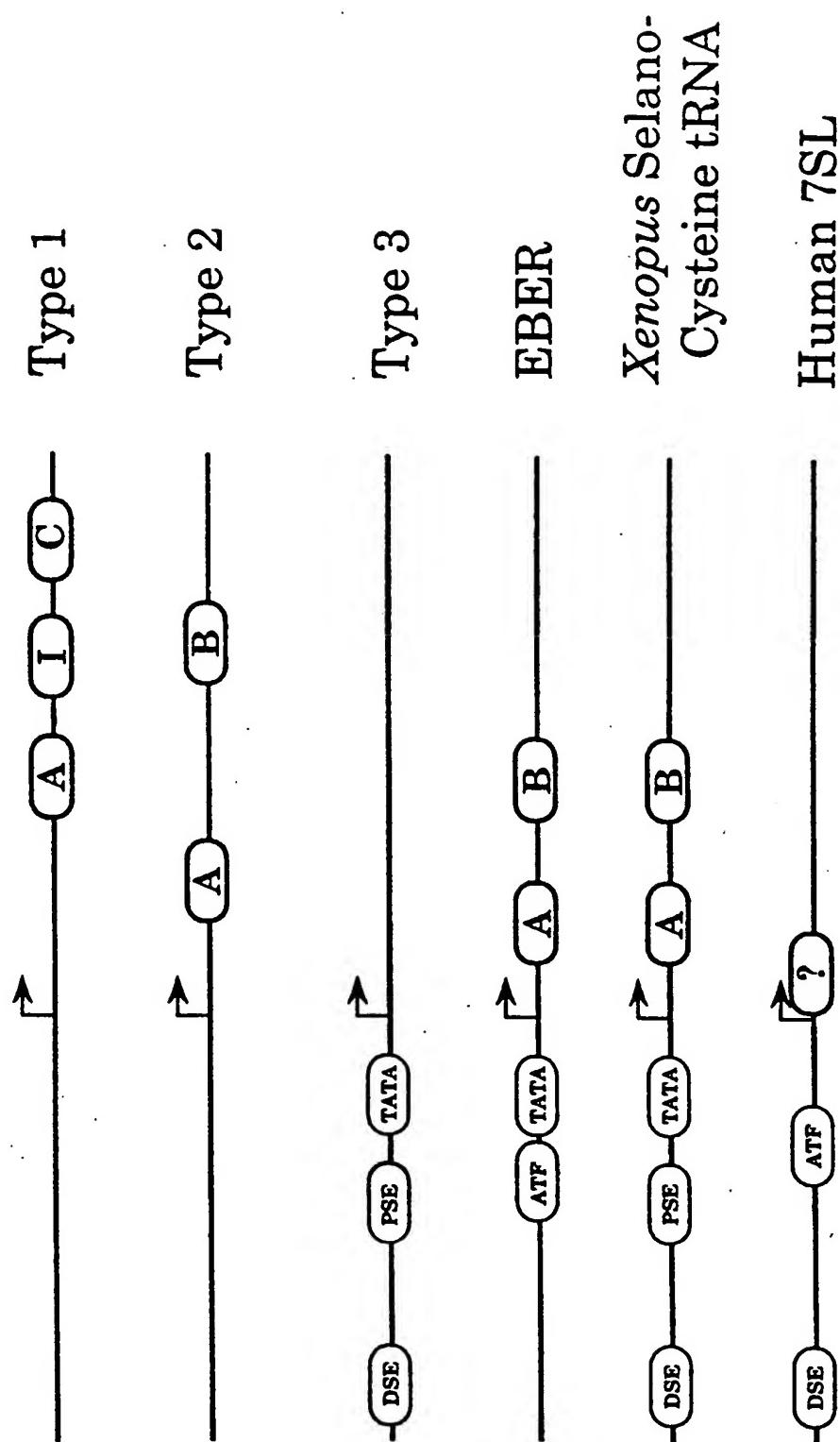


SUBSTITUTE SHEET (RULE 26)

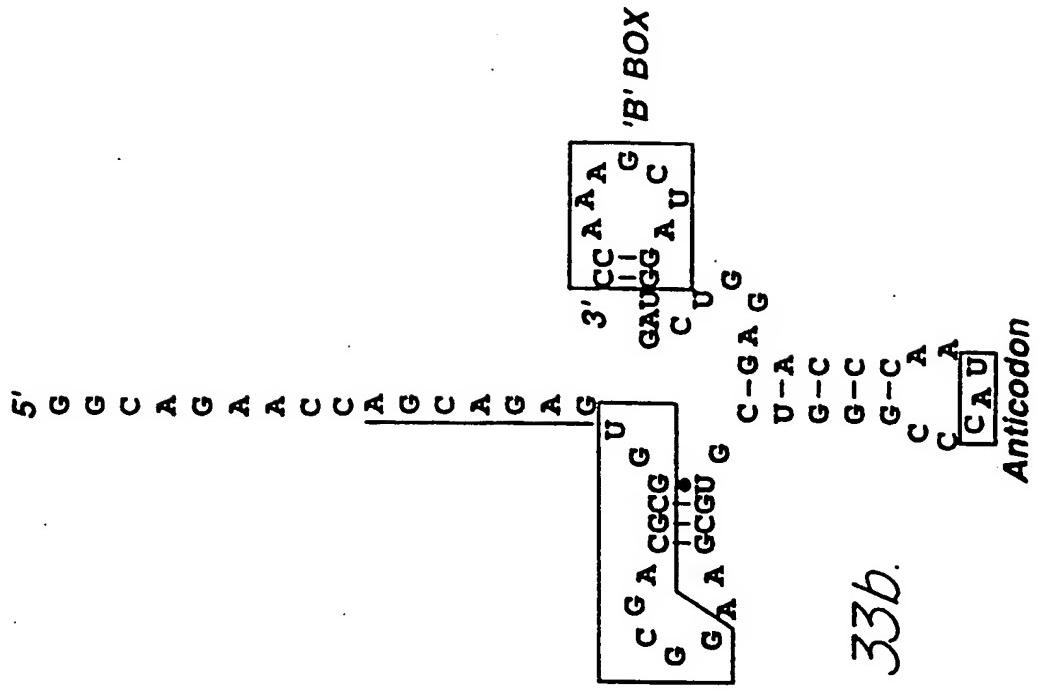
FIG. 31.

31/103

FIG. 32.



32/103



△3-5

FIG. 33b.

tRNA^{met}i

FIG. 33a.

Anticodon

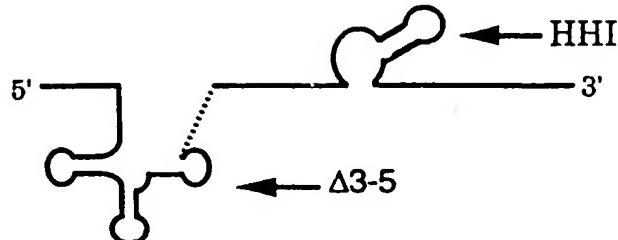
The diagram illustrates the 5' Box RNA structure, which consists of two hairpin loops. The top loop is labeled 'B' BOX and contains the sequence: G-A-U-C-G-A-U-C. The bottom loop is labeled 'A' BOX and contains the sequence: C-G-A-G-C. The two loops are connected by a single G-C base pair.

The diagram illustrates the base pairing between the 3' GGT sequence and the 5' GGCA sequence. The 3' GGT sequence is labeled "Acceptor Stem" and is shown with arrows pointing to the A and T nucleotides. The 5' GGCA sequence is also labeled "Acceptor Stem". The base pairing is as follows:

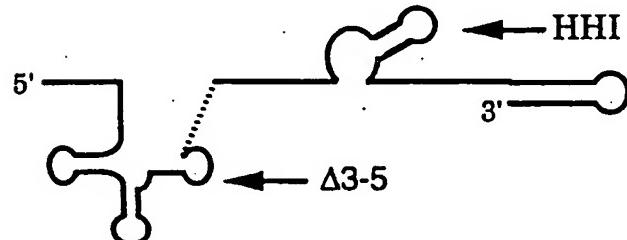
GGT	GGCA
G-C	G-C
G-T	A-T

SUBSTITUTE SHEET (RULE 26)

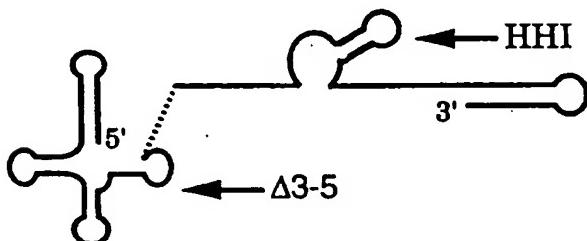
33/103

FIG. 34a. $\Delta 3\text{-}5/\text{HHI}$ *FIG. 34b.*

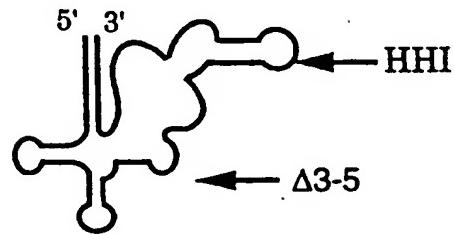
S3

*FIG. 34c.*

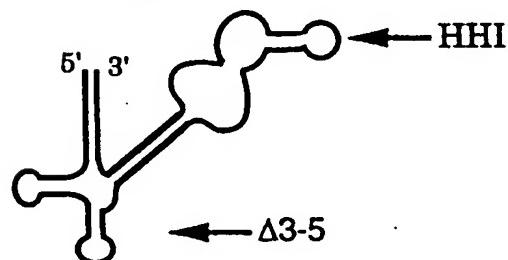
S5

*FIG. 34d.*

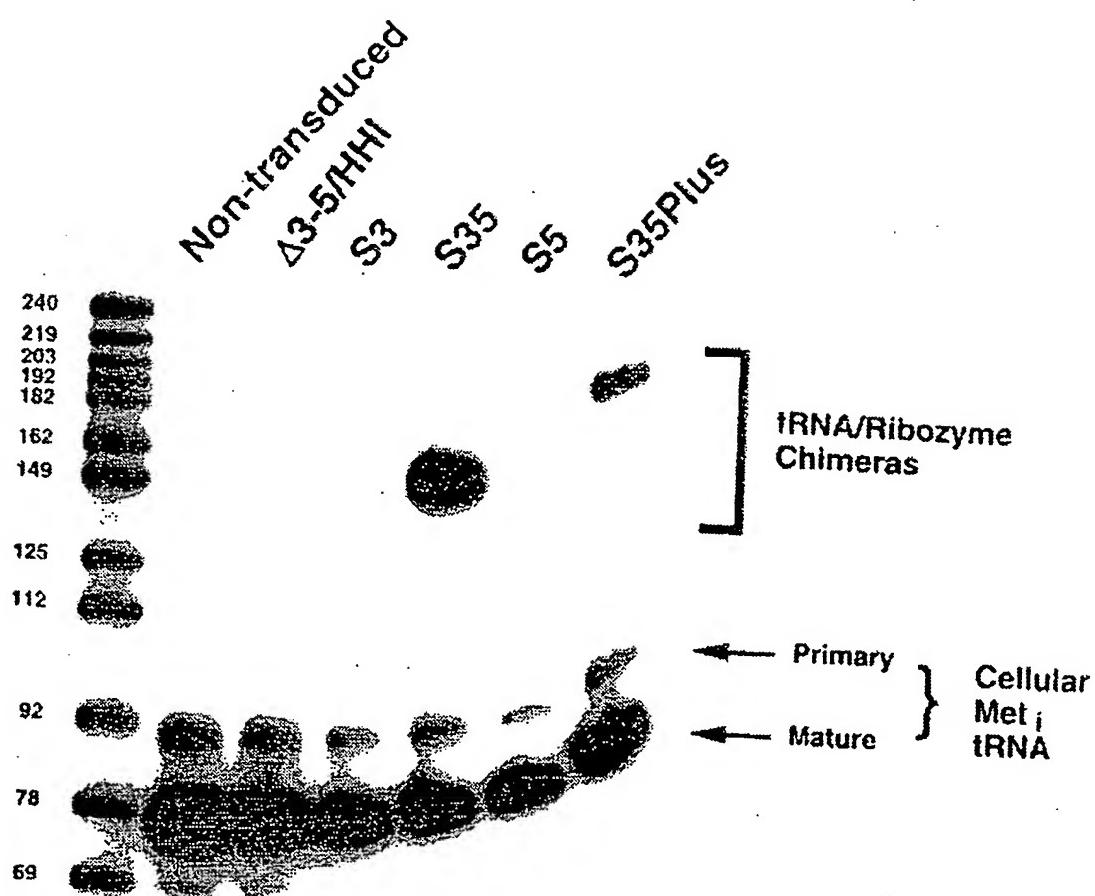
S35

*FIG. 34e.*

S35Plus



34/103



36

FIG. 35.

SUBSTITUTE SHEET (RULE 26)

35/103

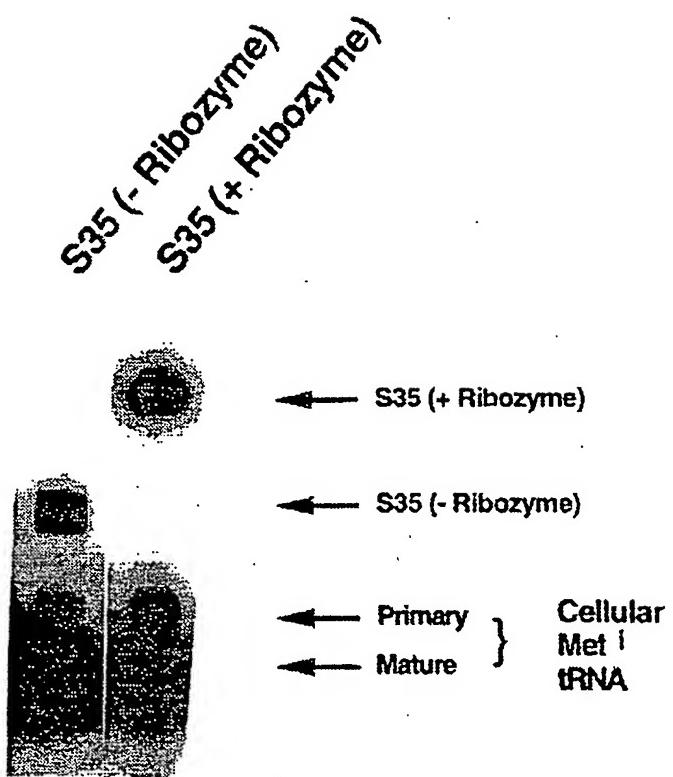


FIG. 36.

36/103

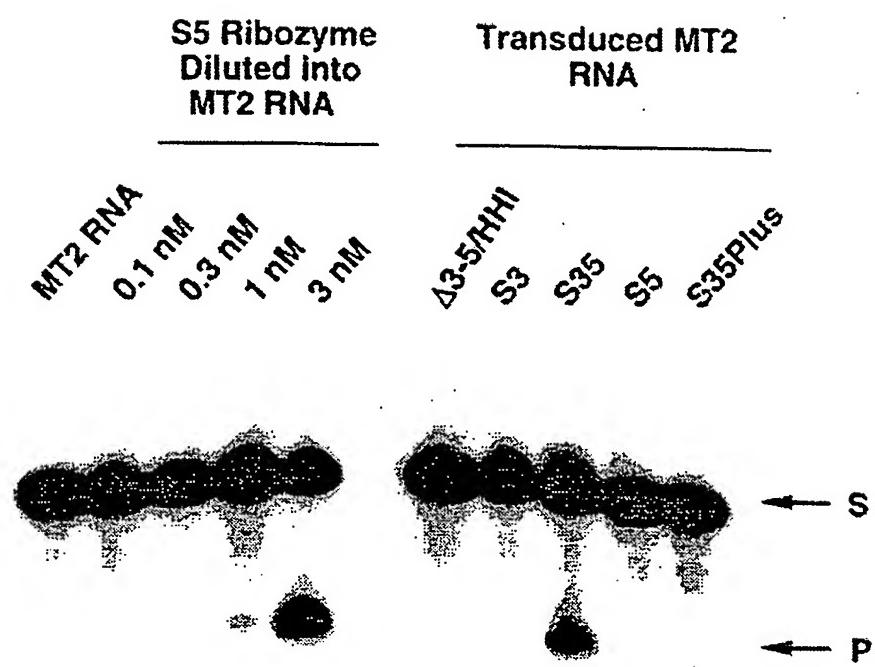
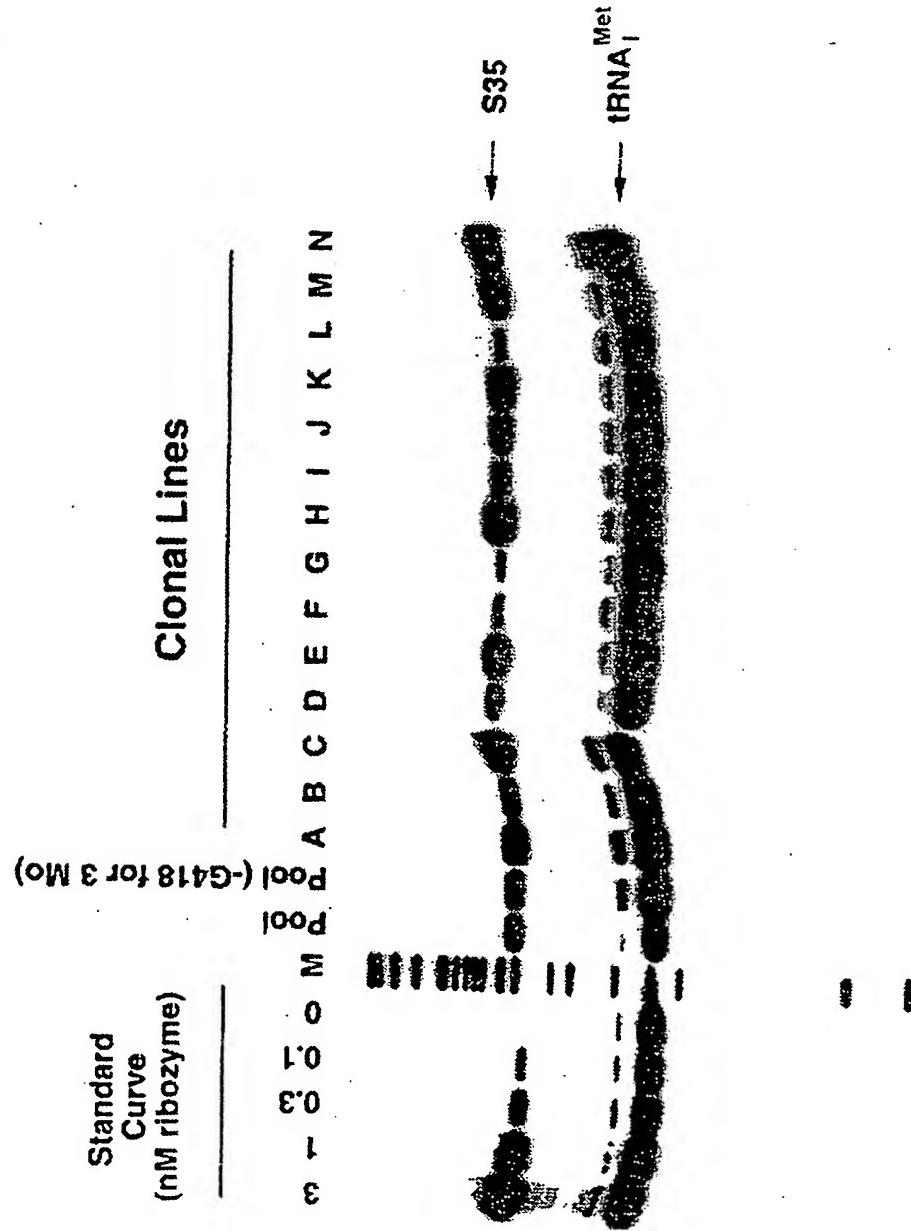


FIG. 37.

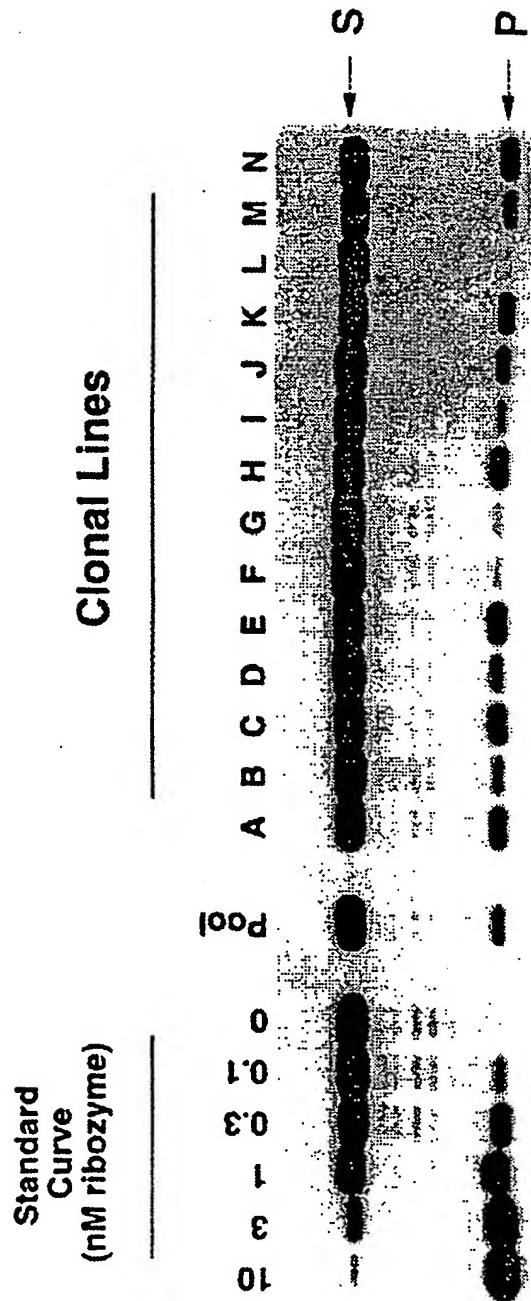
37/103

FIG. 38.



38/103

FIG. 39.



39/103

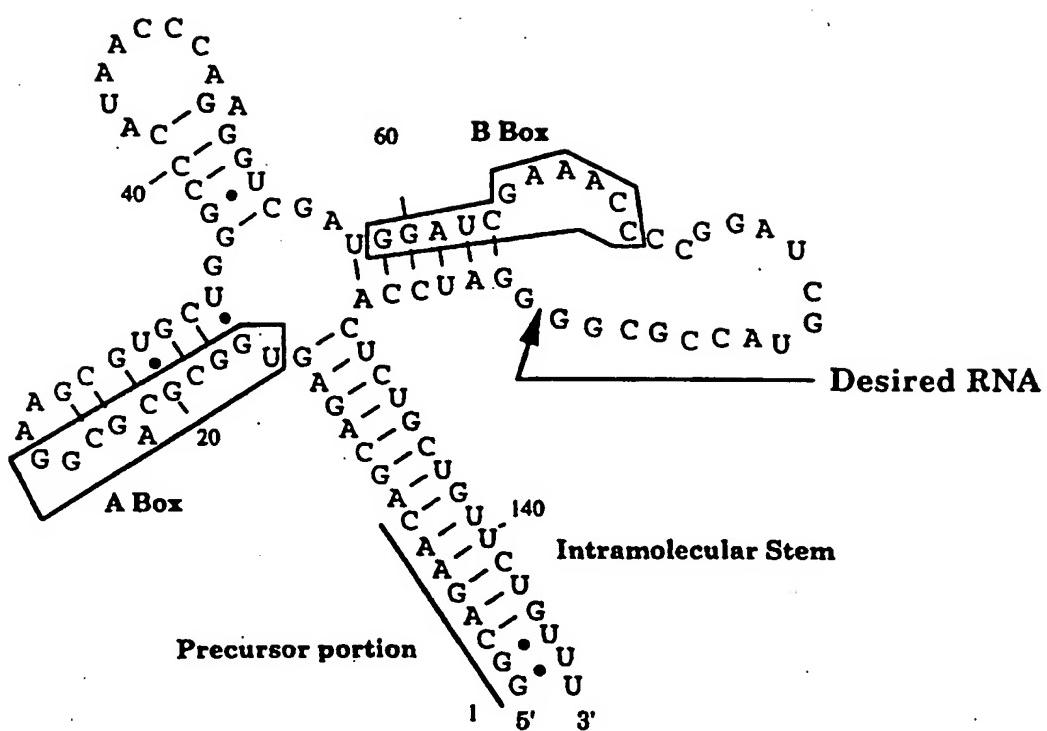


FIG. 40.

40/103

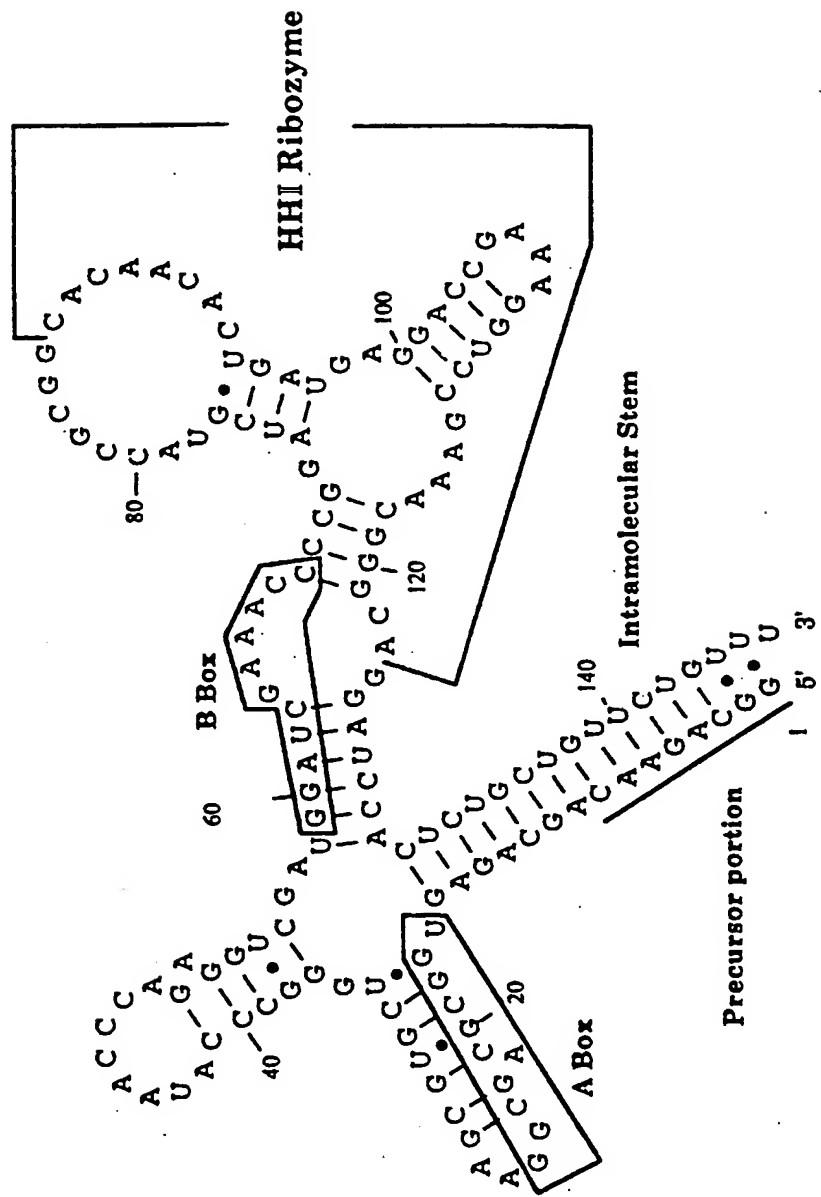


FIG. 41.

41/103

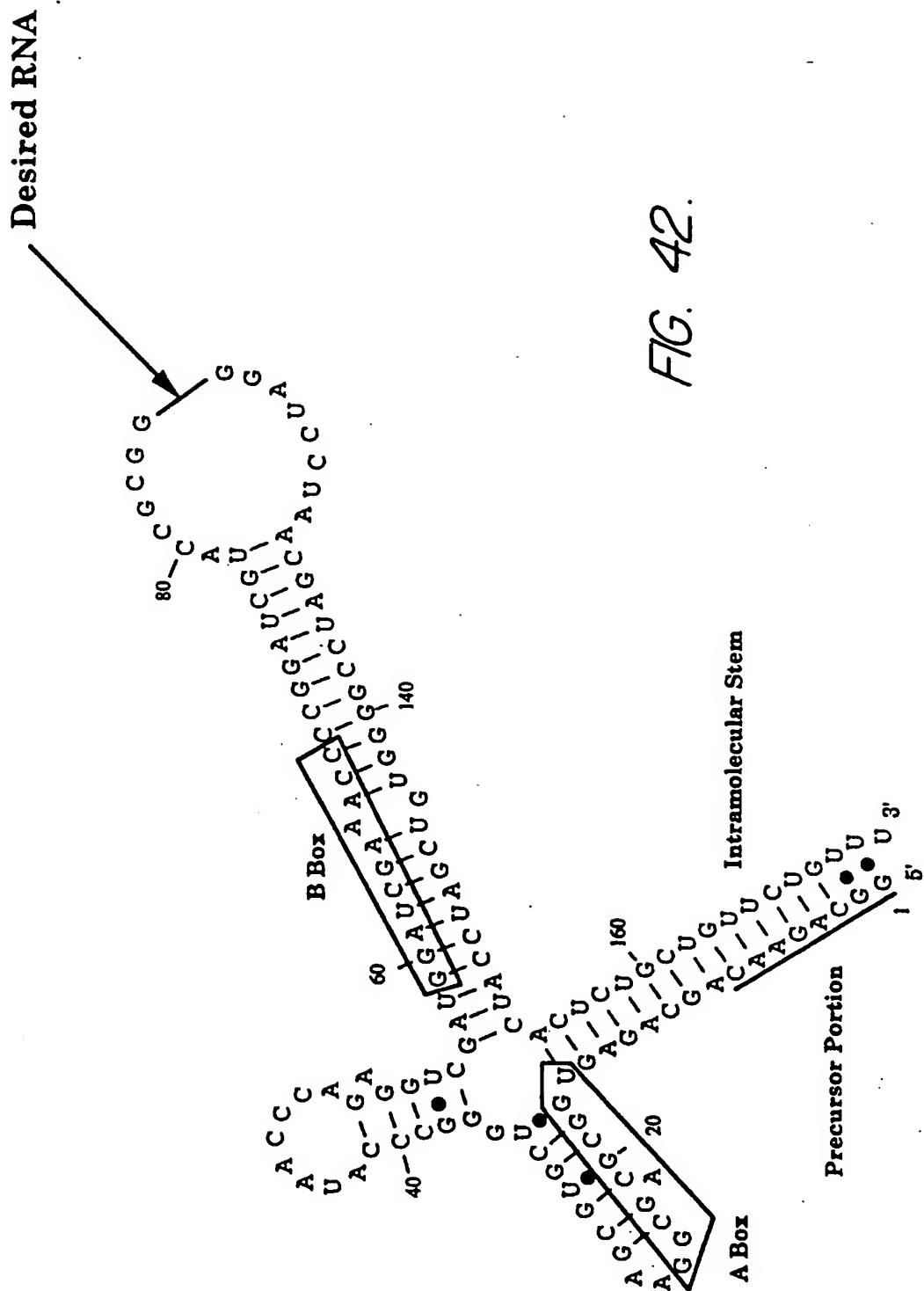


FIG. 42.

SUBSTITUTE SHEET (RULE 26)

42/103

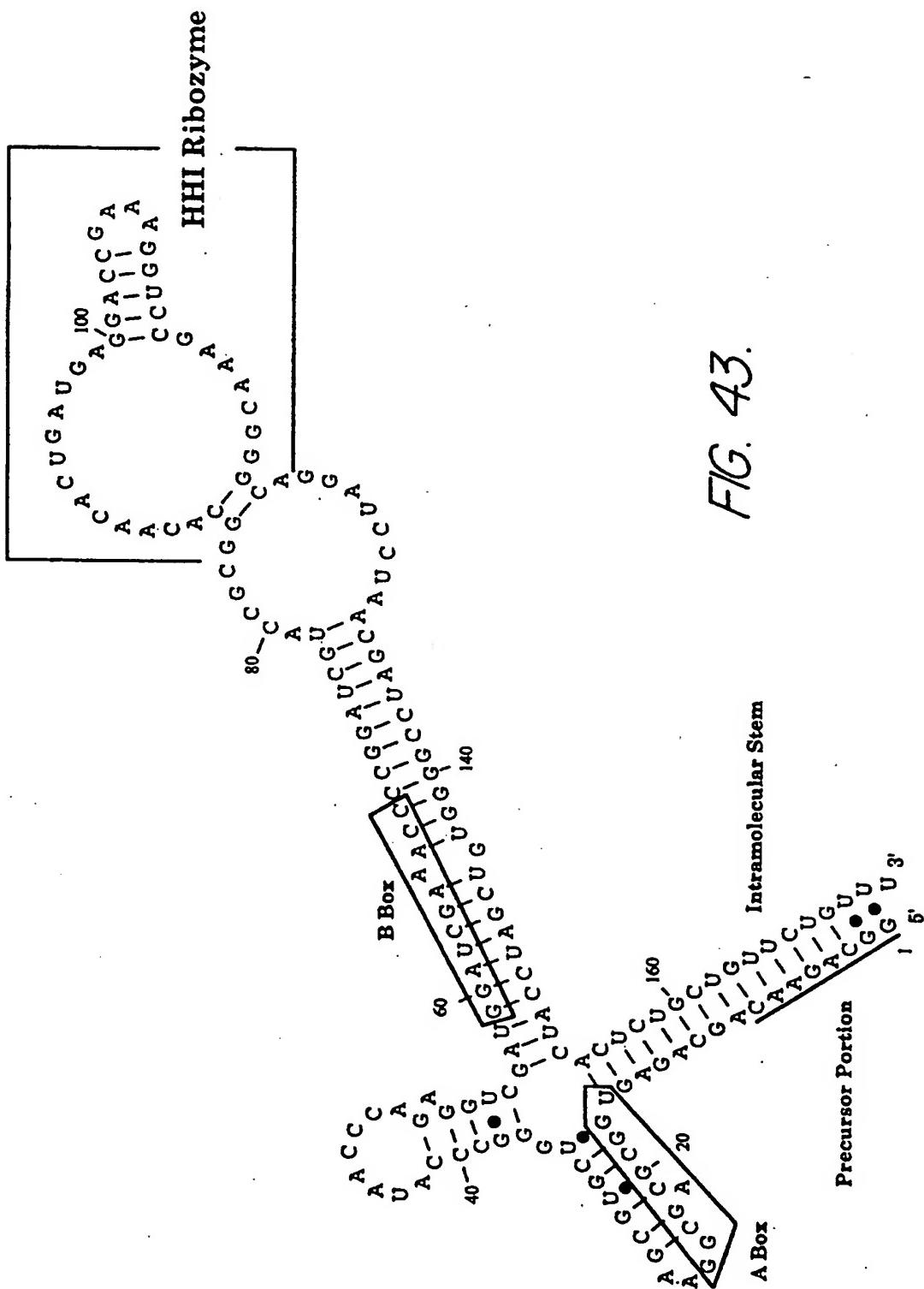


FIG. 43.

43/103

FIG. 44.

S35 Sequence

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG	50
AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGUGGAU CCACUCUGCU	100
GUUCUGUUU	109

FIG. 45.

HHIS35

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG	50
AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGG <u>CACAA</u> <u>CACUGAUGAG</u>	100
<u>GACCGAAAGG UCCGAAACGG GCAGGAUCCA CUCUGCUGUU CUGUUU</u>	146

Underlined bases indicate the HHI ribozyme sequence

FIG. 46.

S35 Plus Sequence

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG	50
AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGGGAUC CUAACGAUCC	100
GGGGUGUCGA UCCAUCACUC UGCUGUUUCUG UU U	133

FIG. 47.

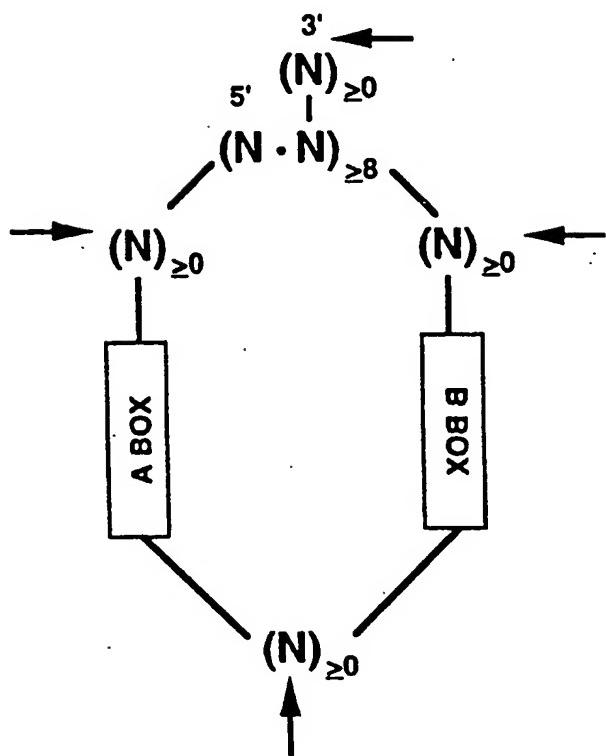
HHIS35 Plus

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG	50
AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGG <u>CACAA</u> <u>CACUGAUGAG</u>	100
<u>GACCGAAAGG UCCGAAACGG GCAGGAUCCU AACGAUCCGG GGUGUCGAUC</u>	150
CAUCACUCUG CUGUUUCUGUU U	171

Underlined bases indicate the HHI ribozyme sequence
SUBSTITUTE SHEET (RULE 26)

44/103

FIG. 48.



A BOX = URGCNNAGYGG

B BOX = GGUUCGANUCC

This is based on Geiduschek & Tocchini-Valentini,
 (1988) *Annu. Review Biochem.* 57, 873-914. However
 this consensus sequence is not meant to be limiting

N = A, U, G, or C

R = Purine

Y = Pyrimidine

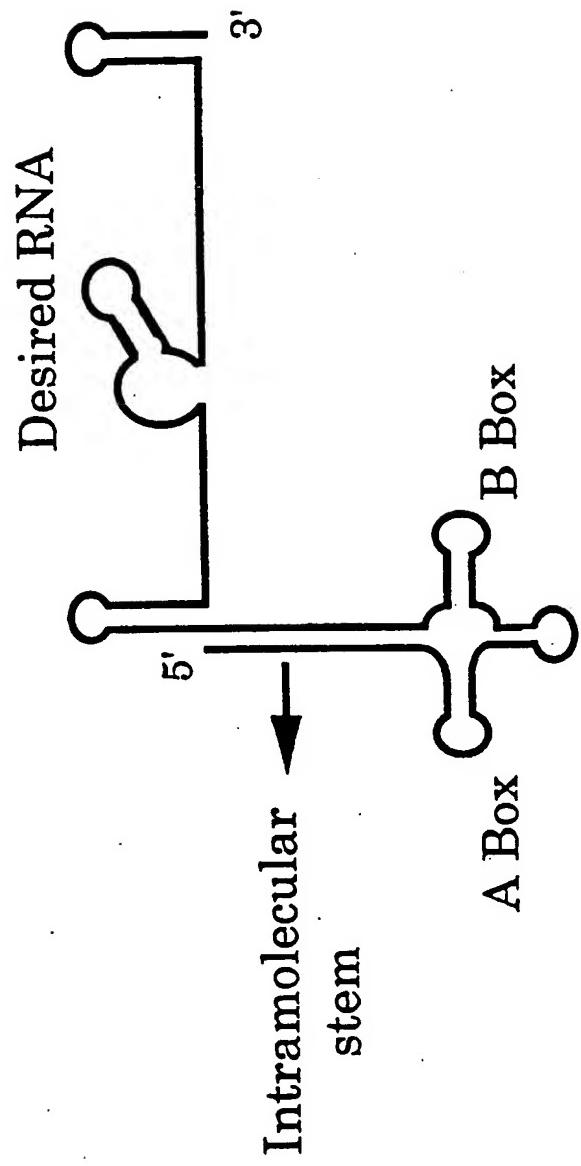
• = Indicates base-pairing

— = Indicates covalent linkage

→ = Indicates sites at which desired
RNAs can be cloned

45/103

FIG. 49.



46/103

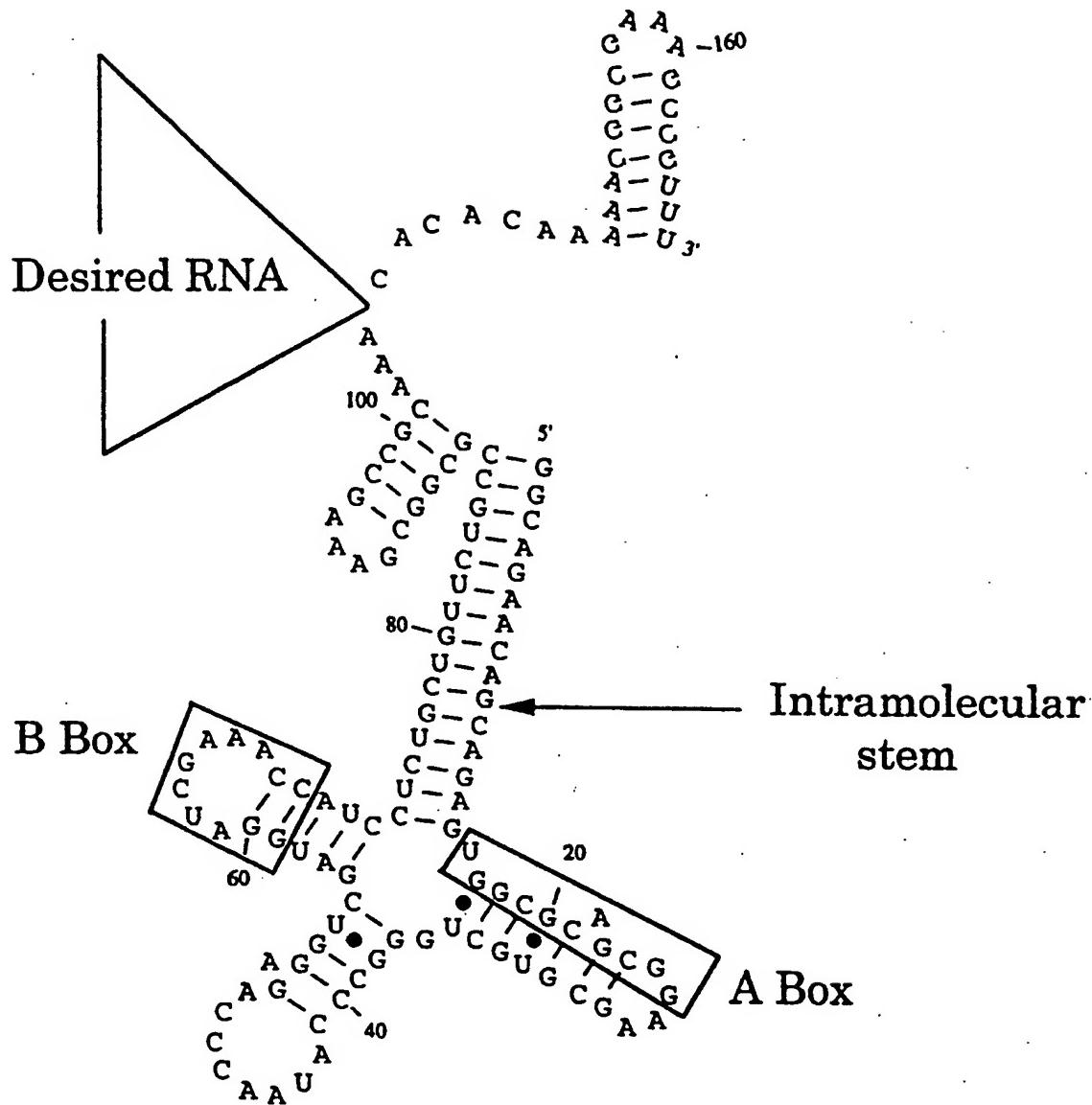


FIG. 50.

47/103

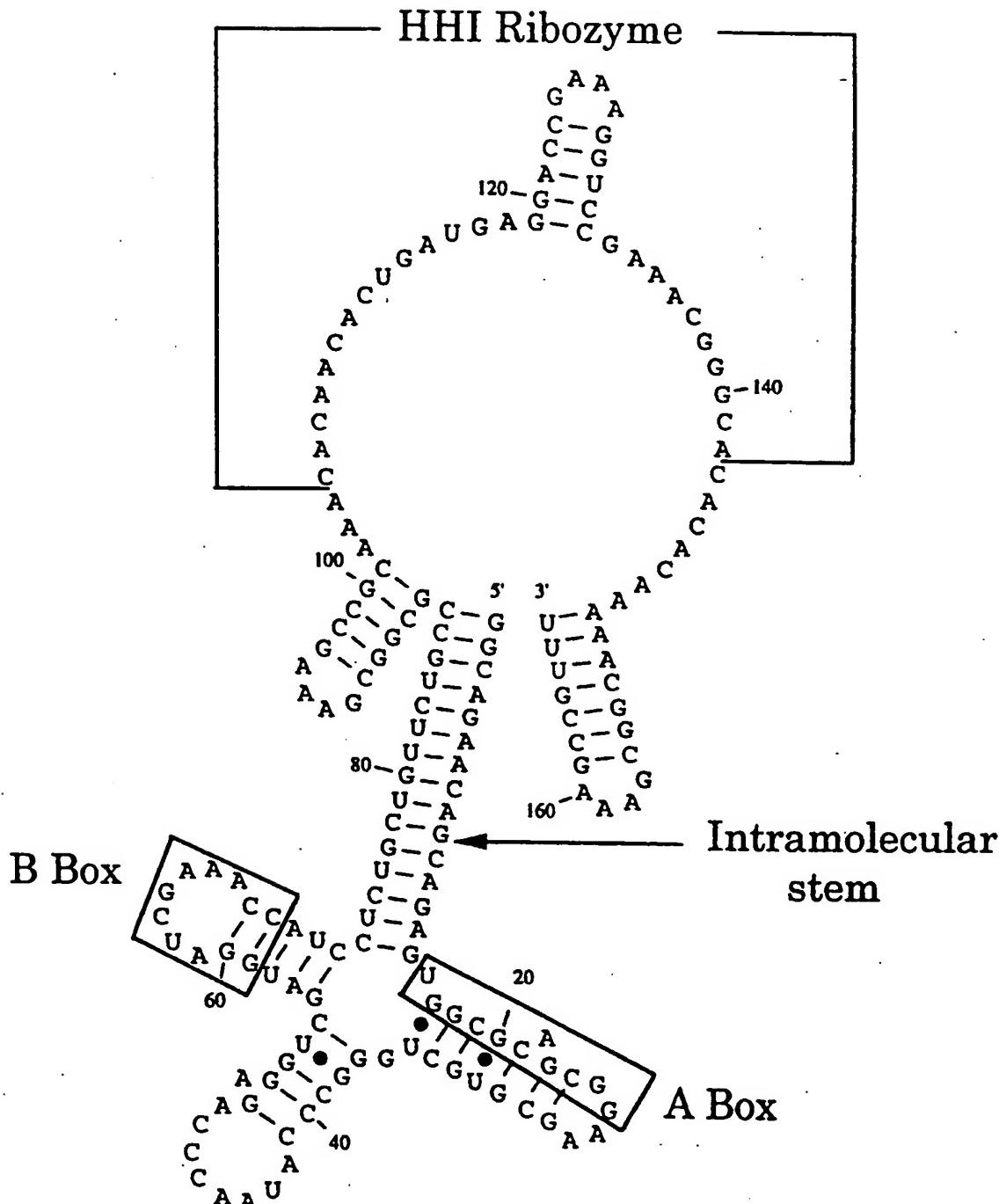
*FIG. 51.*

FIG. 52a.

48/103

A: TRZ-A

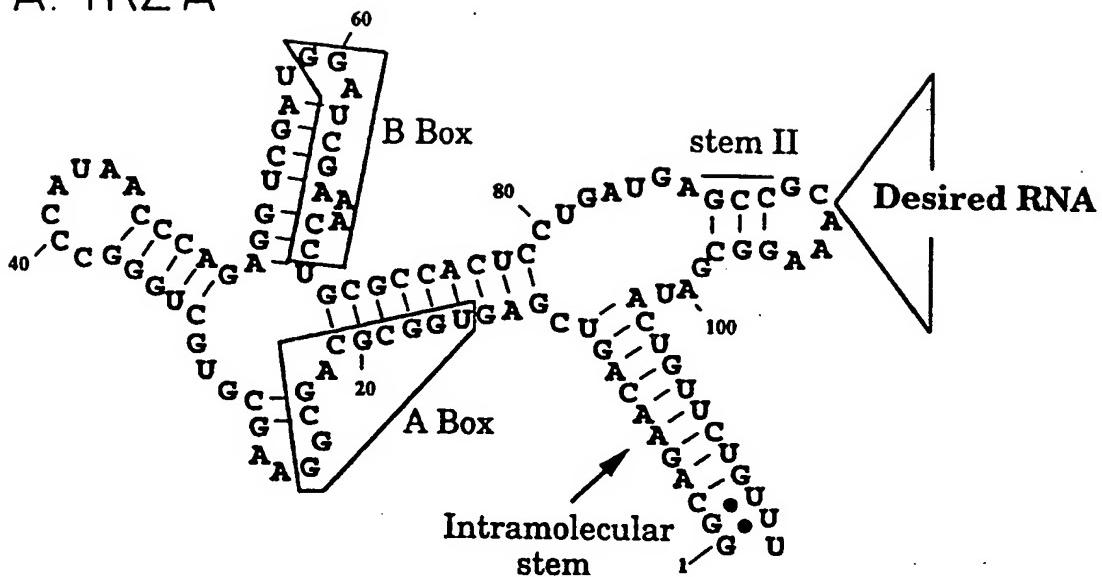
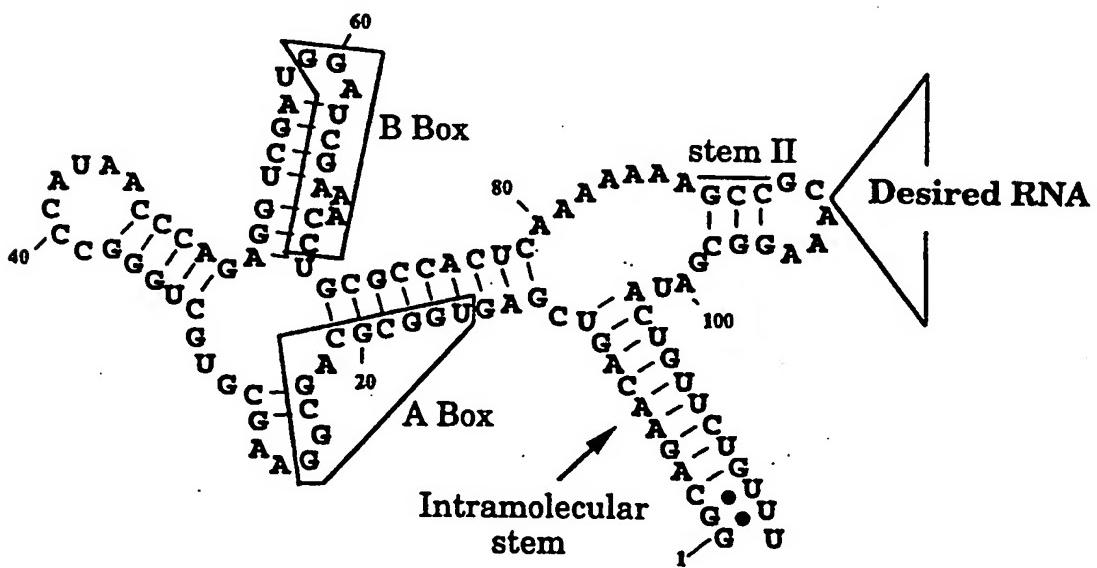


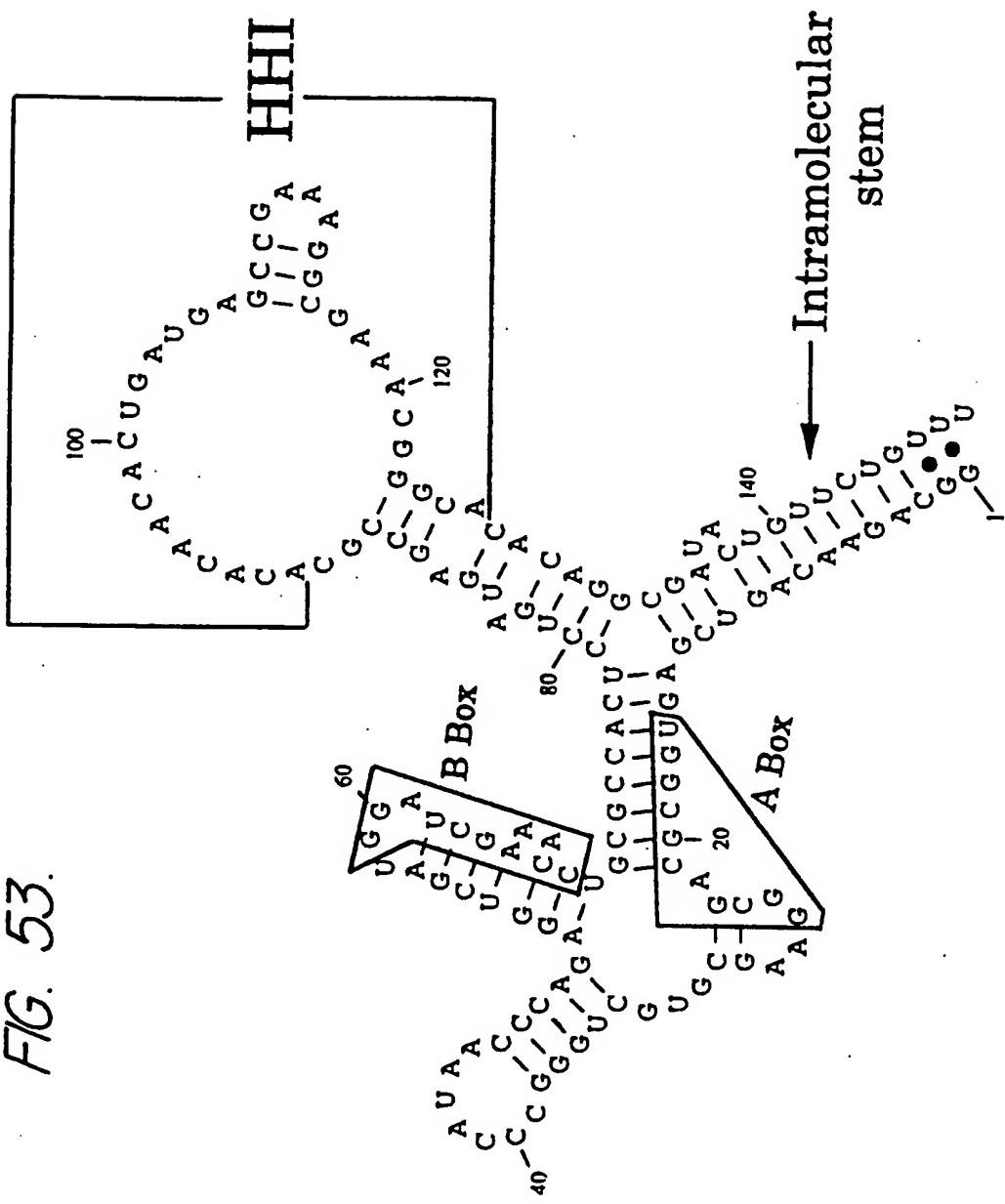
FIG. 52b.

B: TRZ-B



49/103

FIG. 53.



50/103

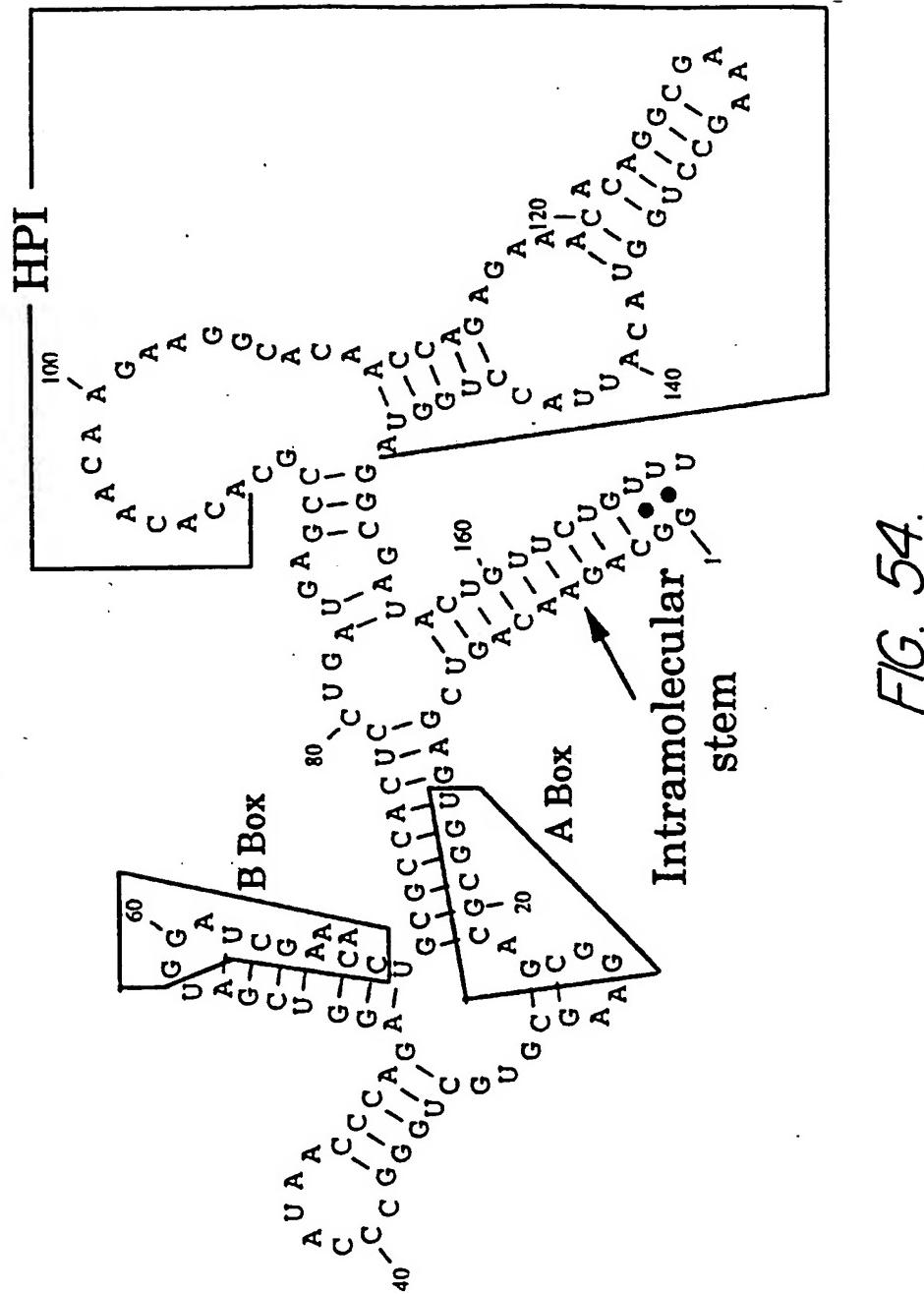


FIG. 54.

51/103

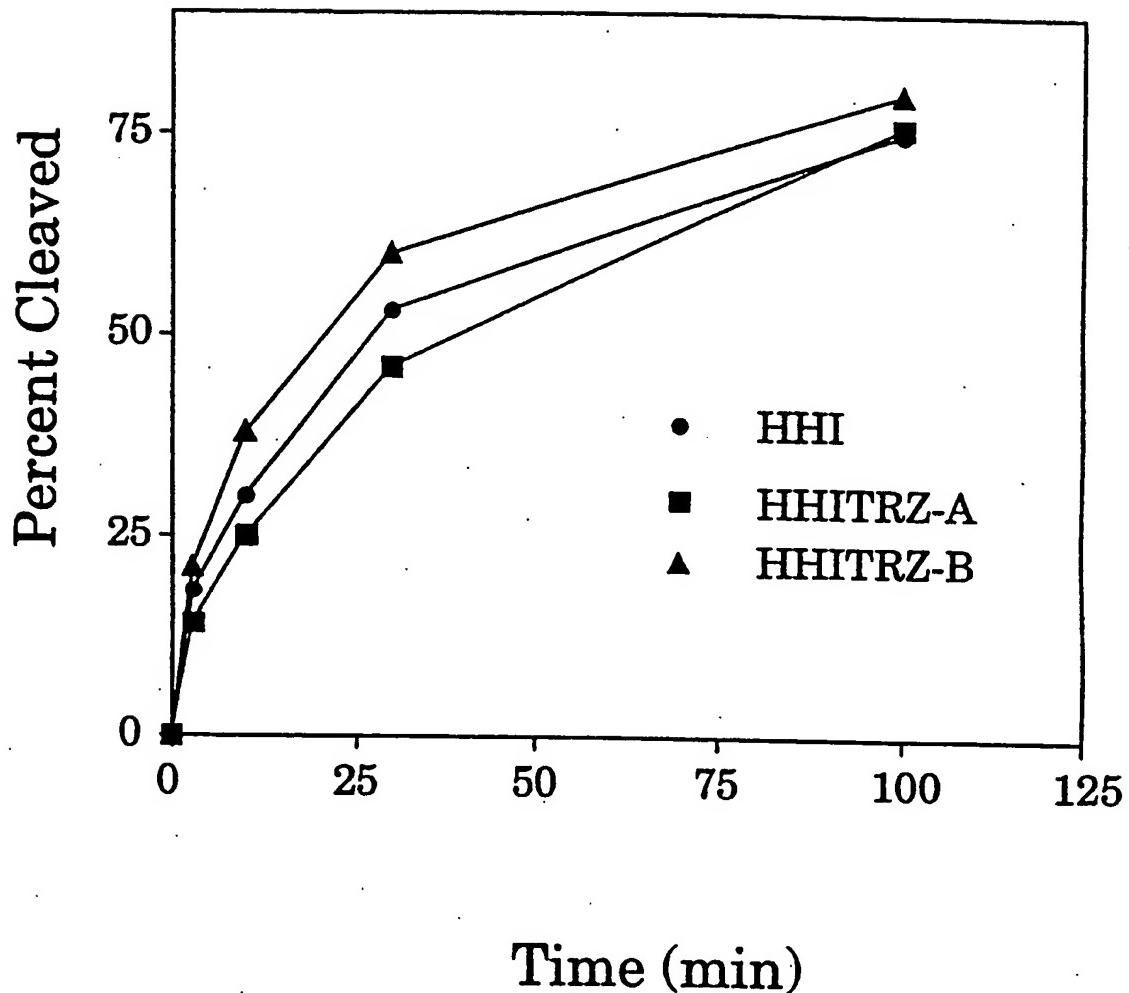


FIG. 55.

52/103

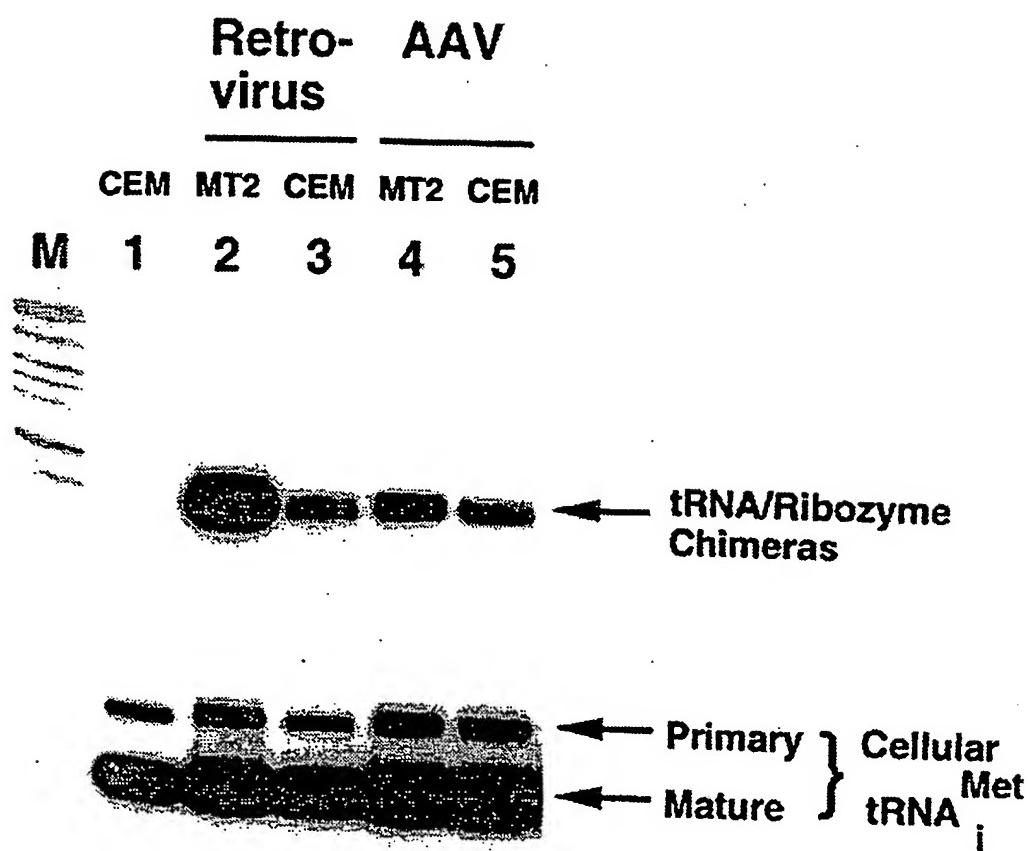
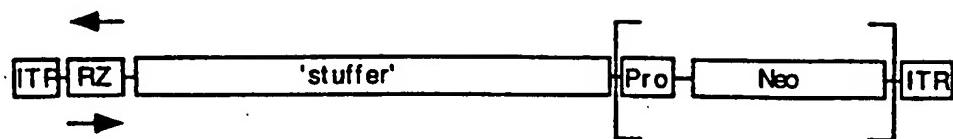


FIG. 56.

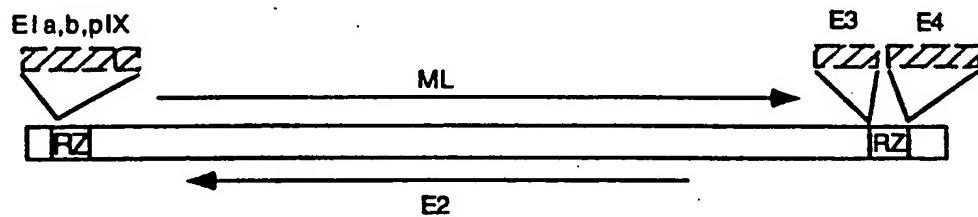
53/103

FIG. 57a.

AAV Vector

*FIG. 57b.*

Adenovirus Vector



54/103

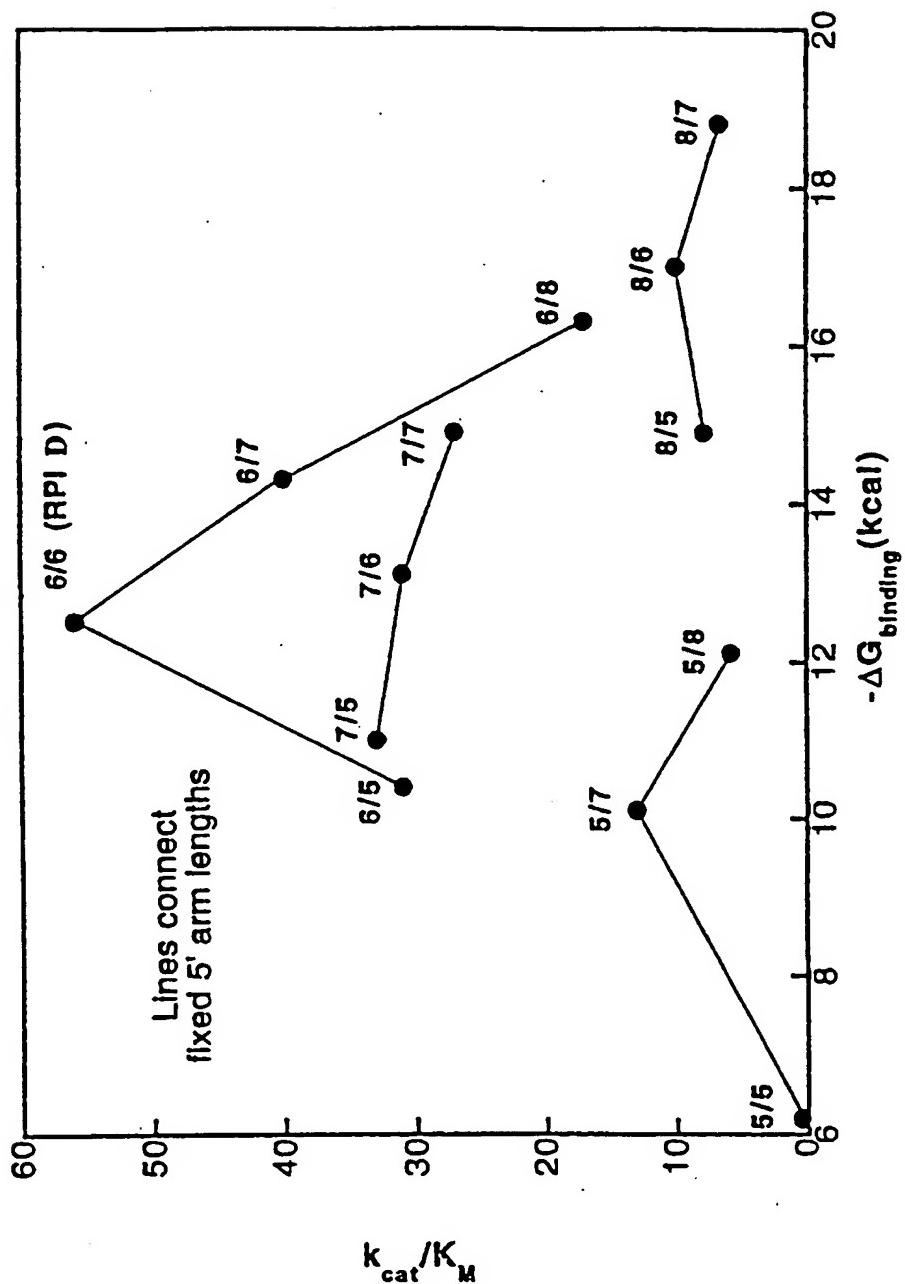


FIG. 58.

55/103

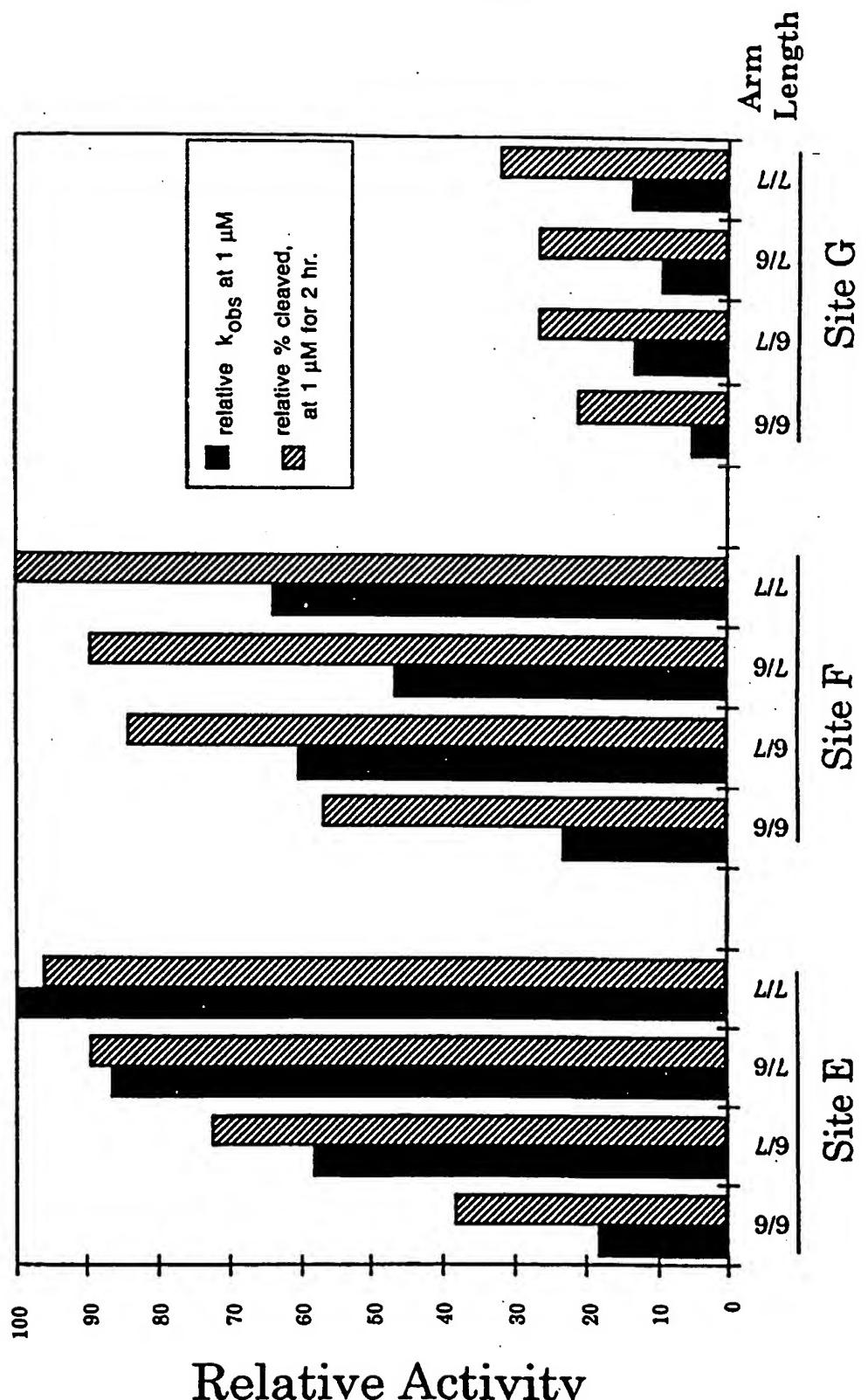
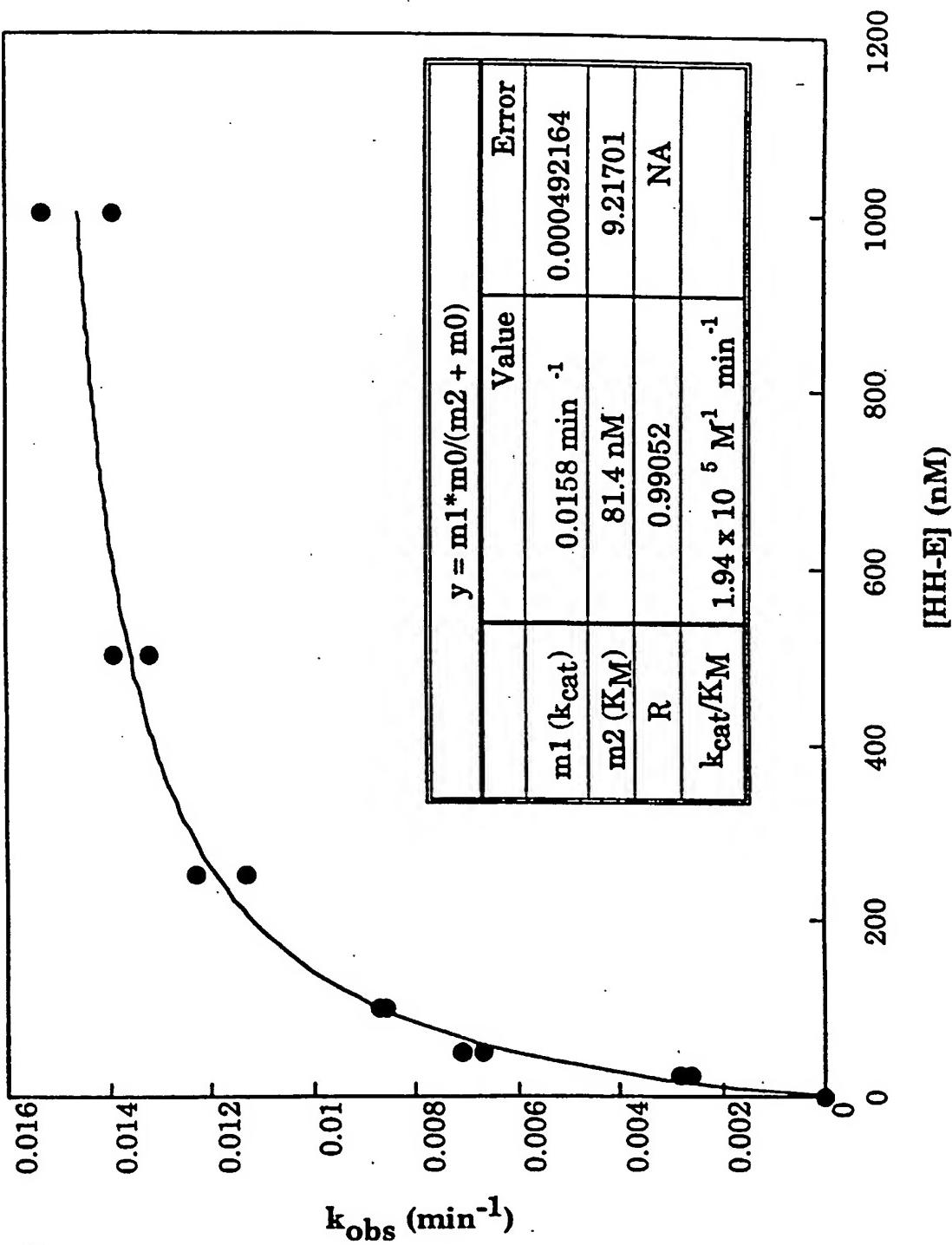


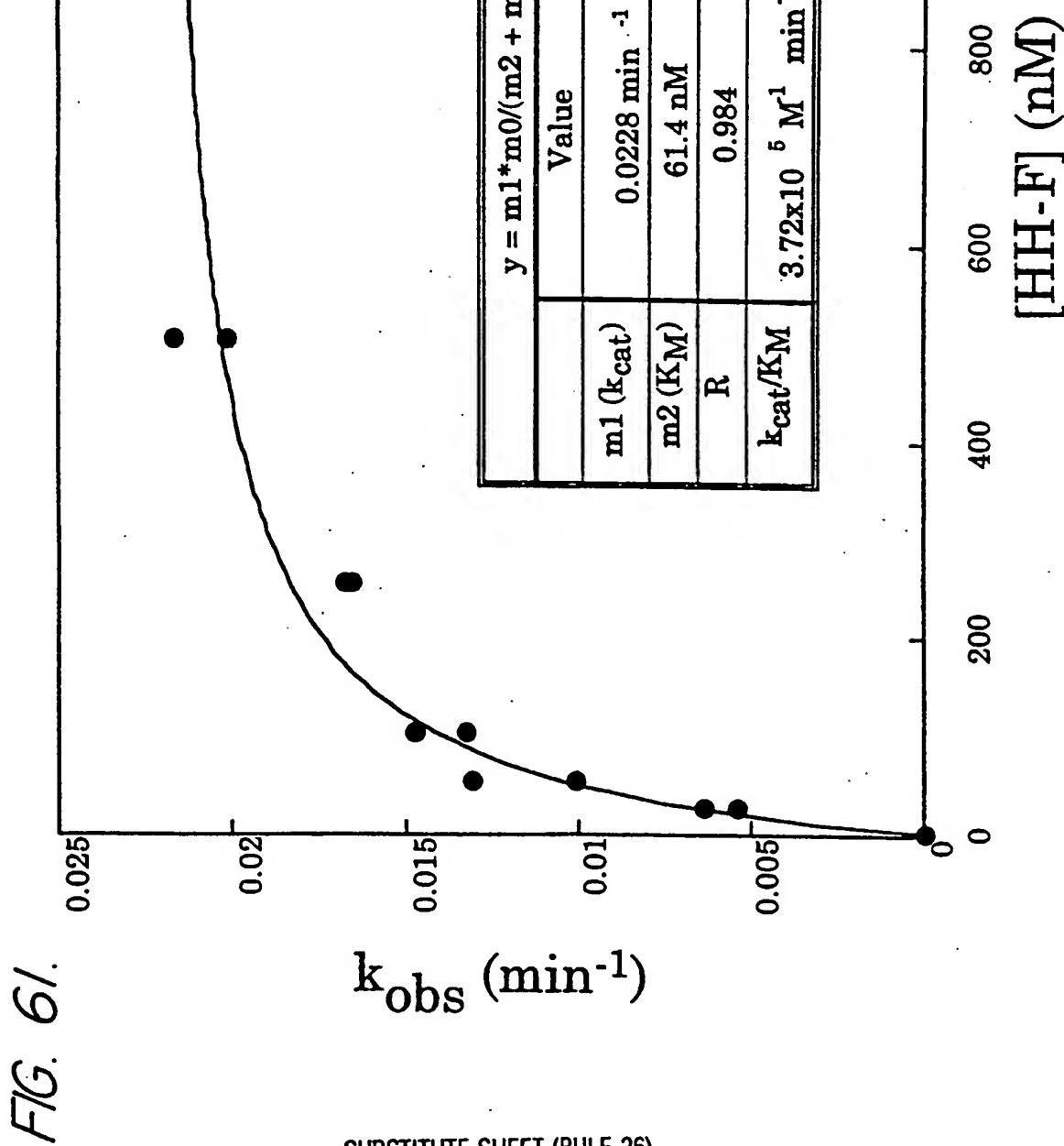
FIG. 59.

Ribozyme

56/103



57/103



58/103

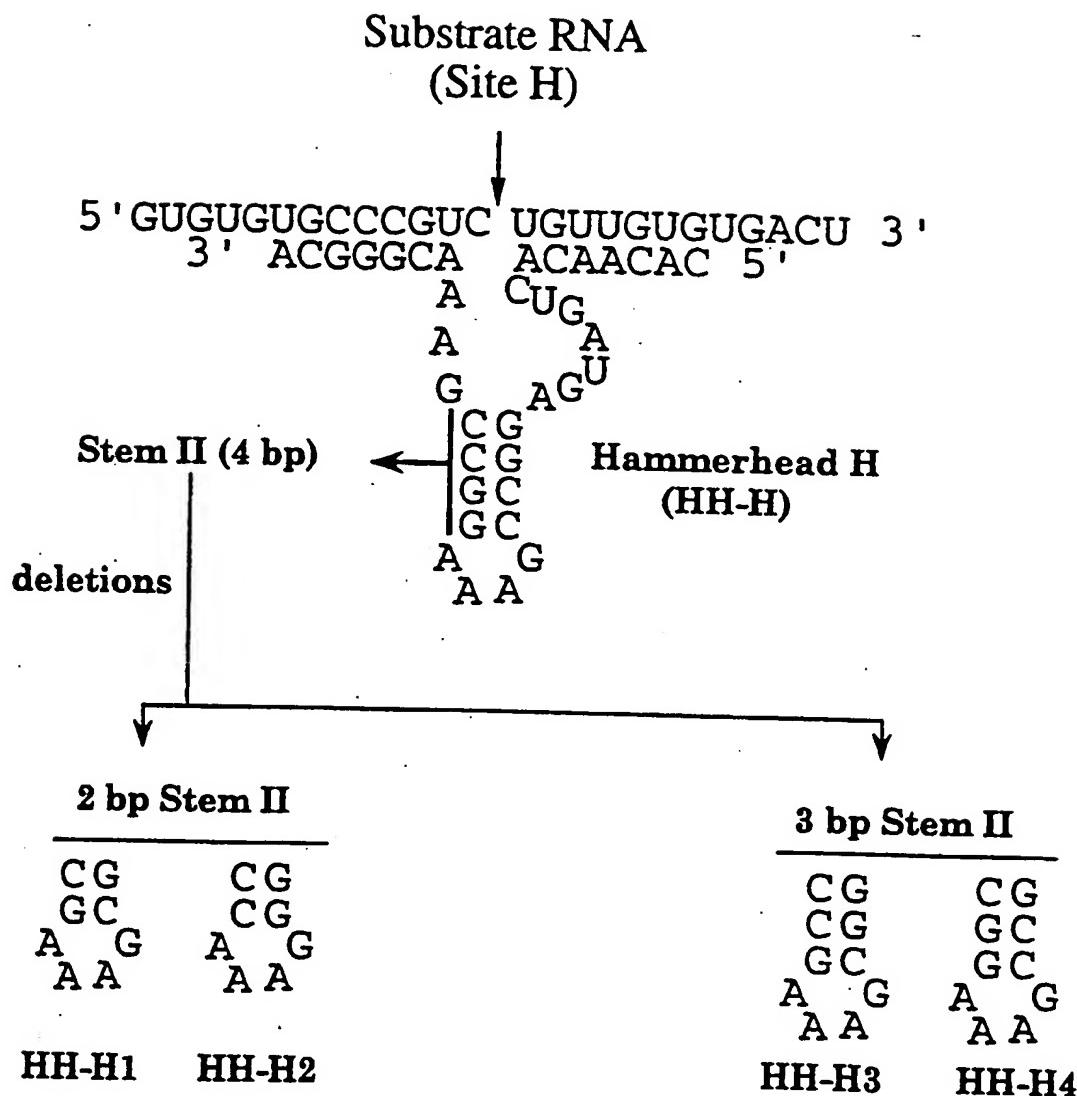


FIG. 62.

59/103

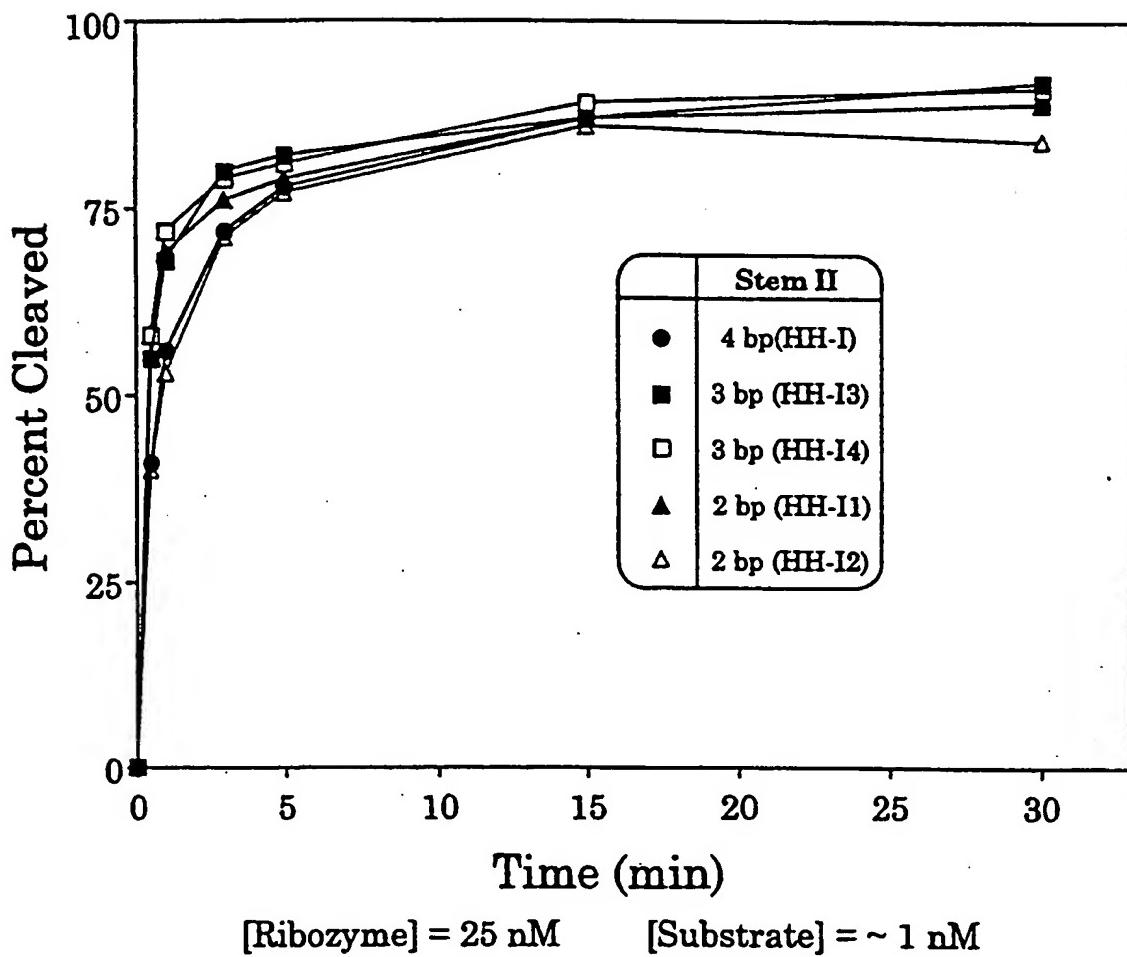


FIG. 63.

60/103

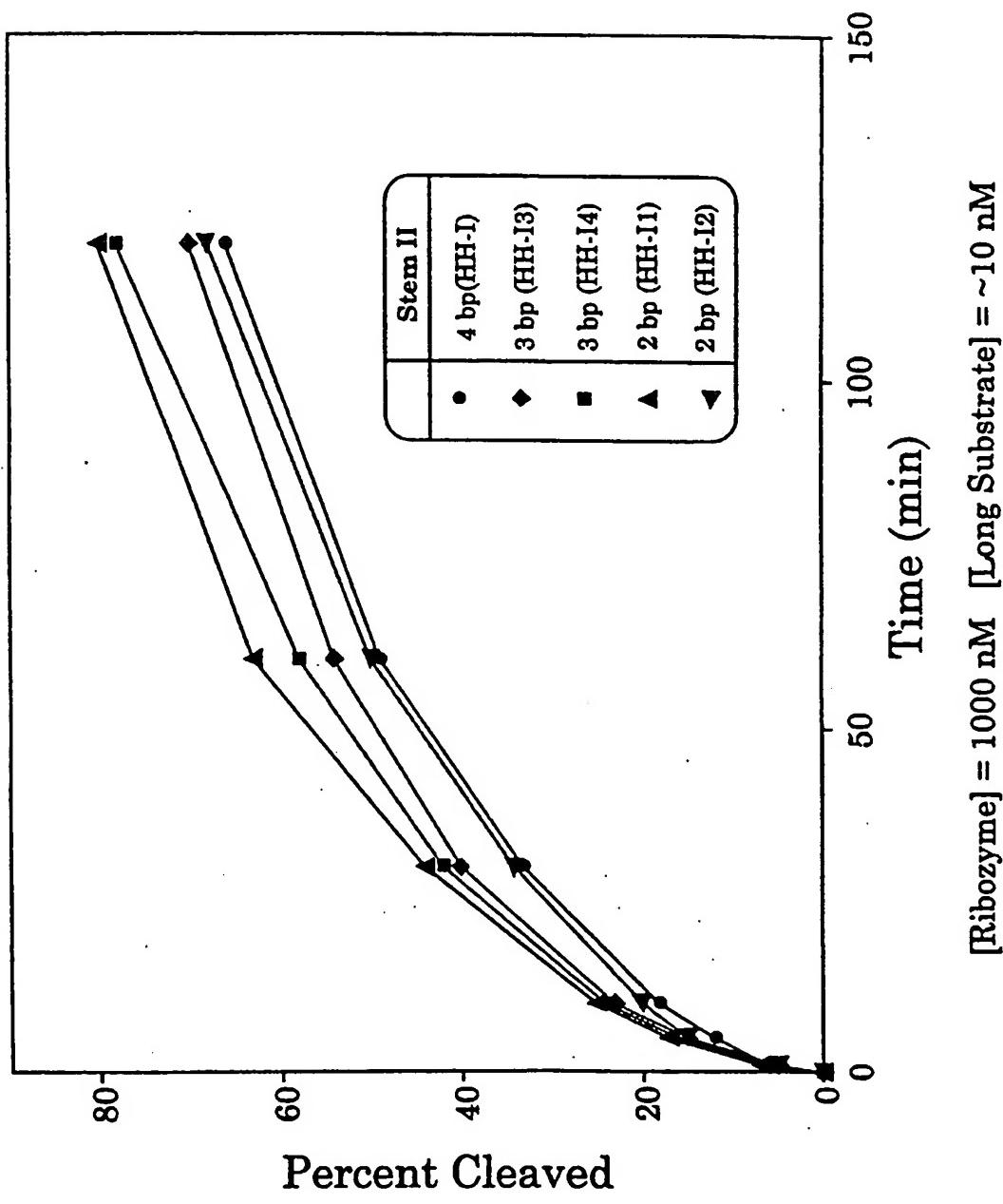


FIG. 64.

FIG. 65a.

Substrate RNA (site J)

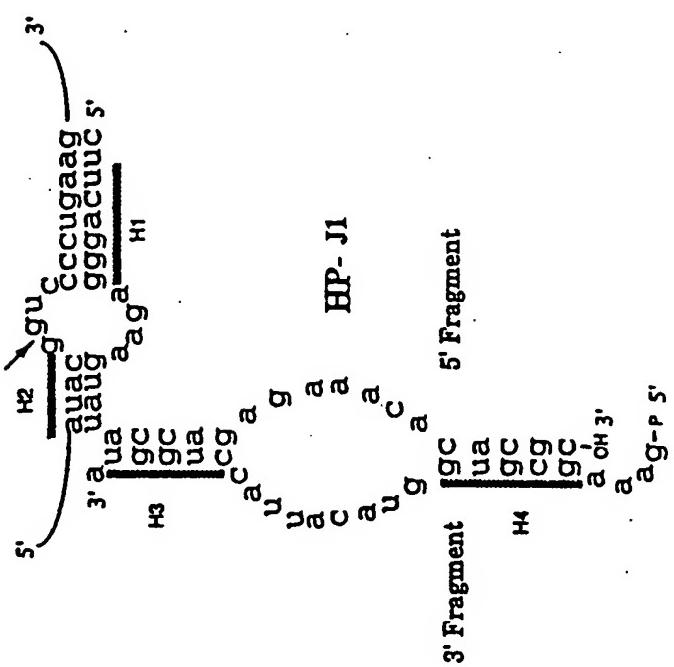
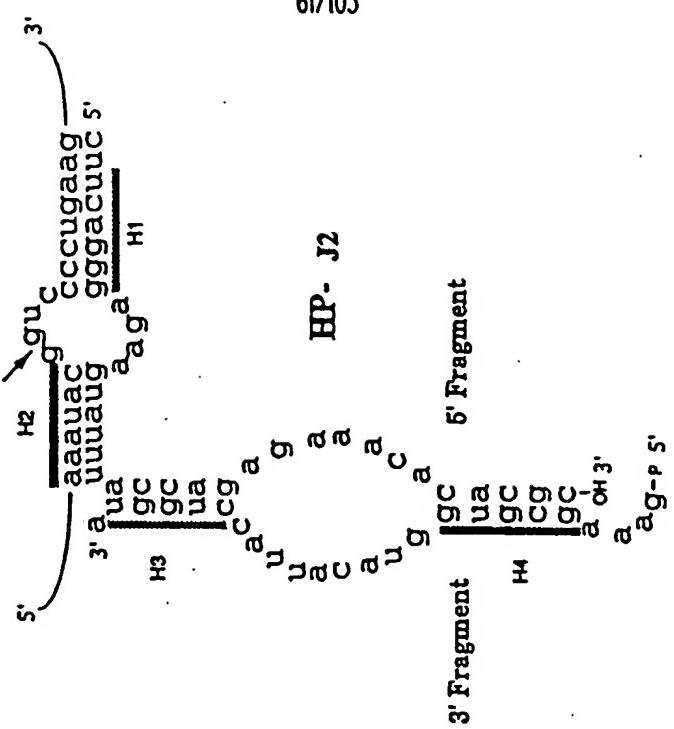


FIG. 65b.

Substrate RNA (site J)



61/103

62/103

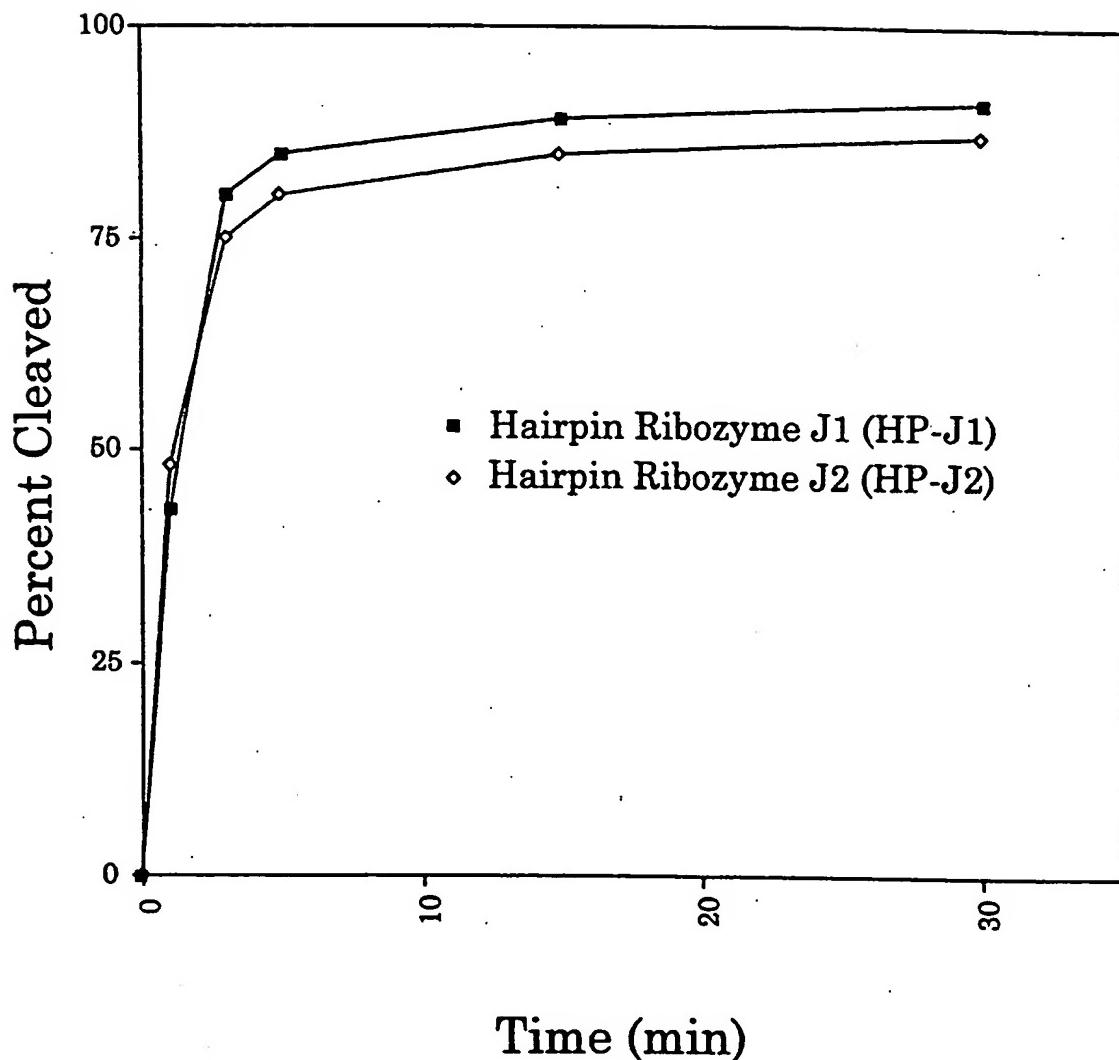


FIG. 66.

FIG. 67a.

Substrate RNA

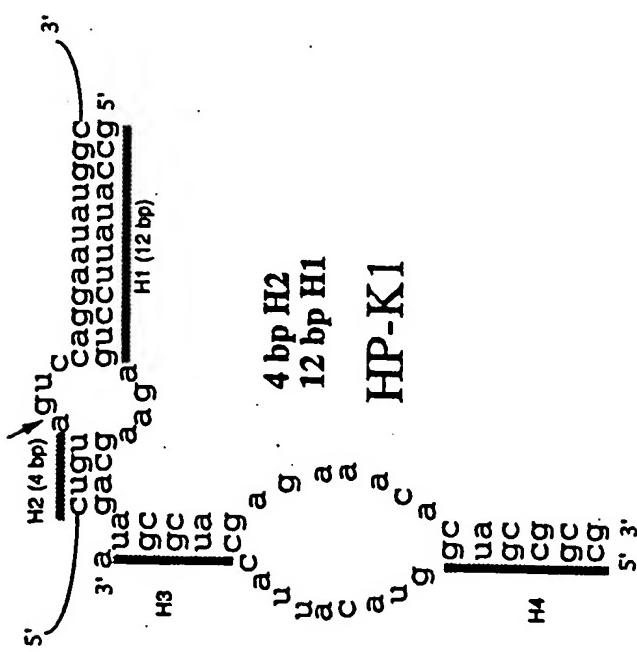
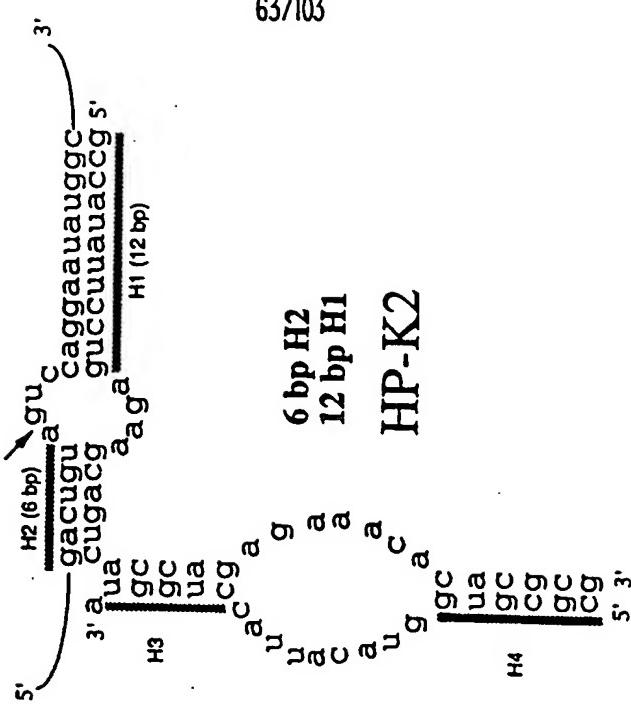


FIG. 67b.

Substrate RNA



64/103

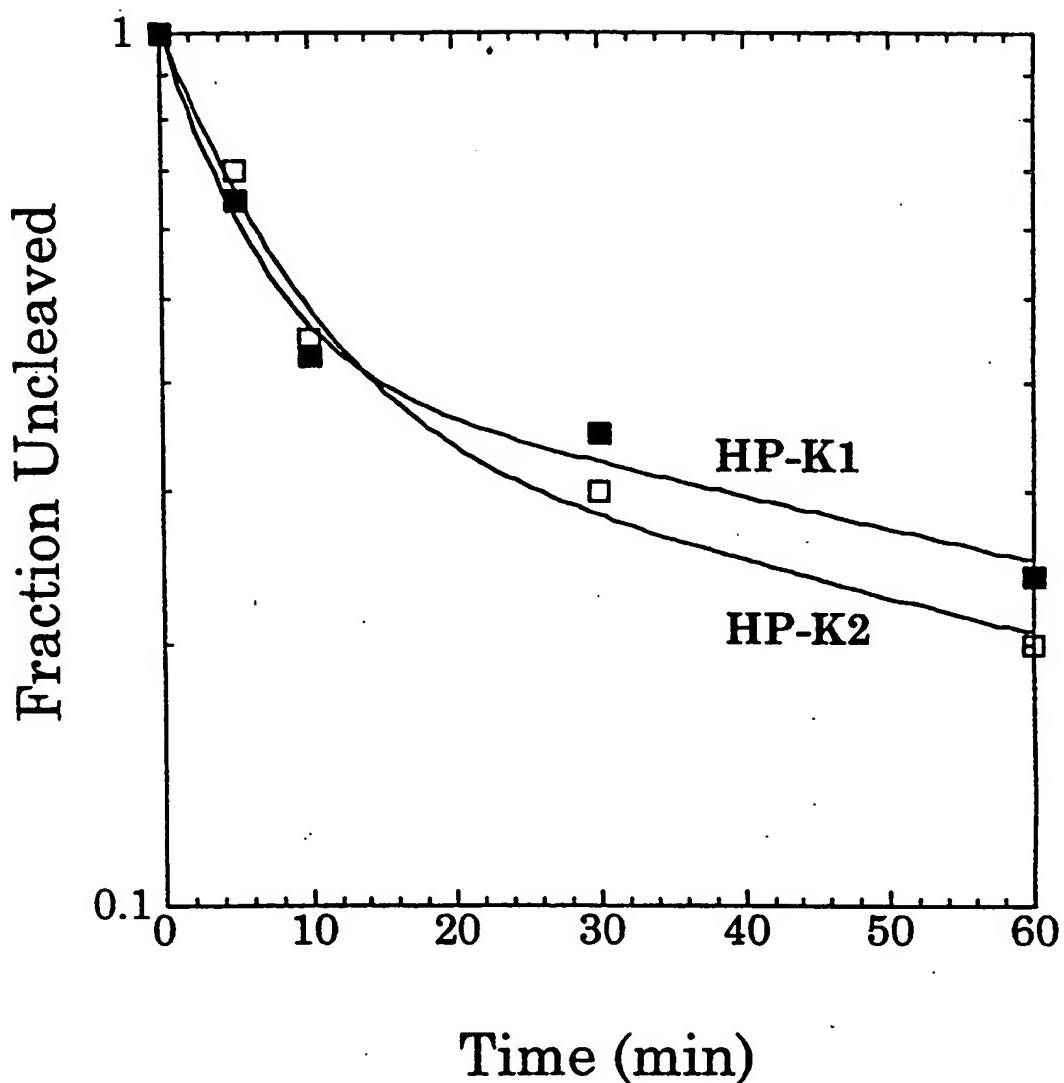


FIG. 68.

FIG. 69a.

Substrate RNA

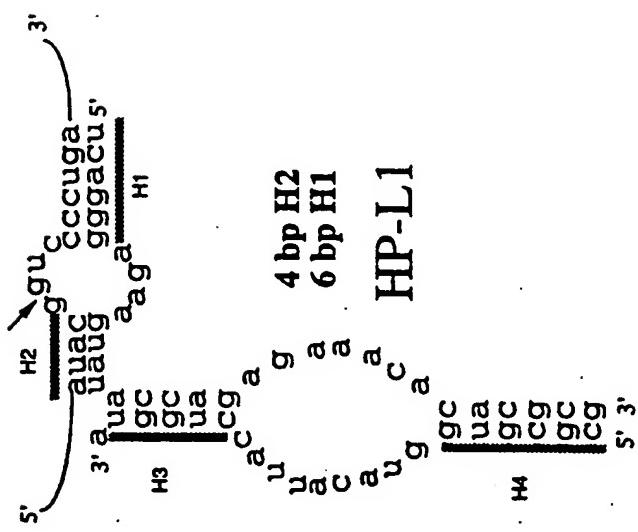
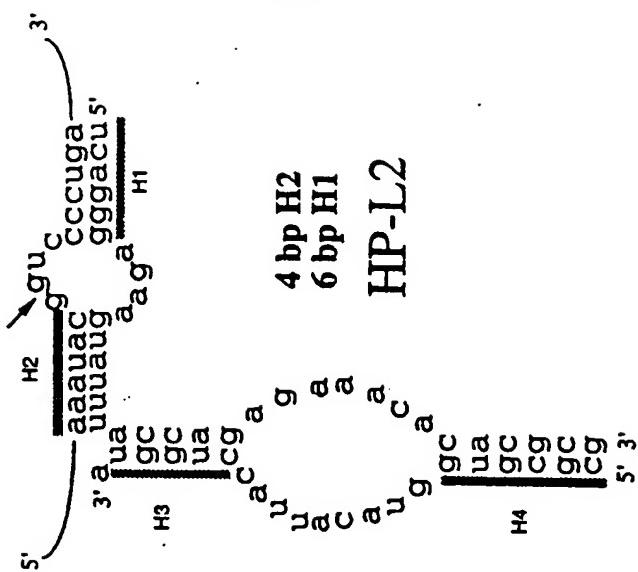


FIG. 69b.

Substrate RNA



65/103

66/103

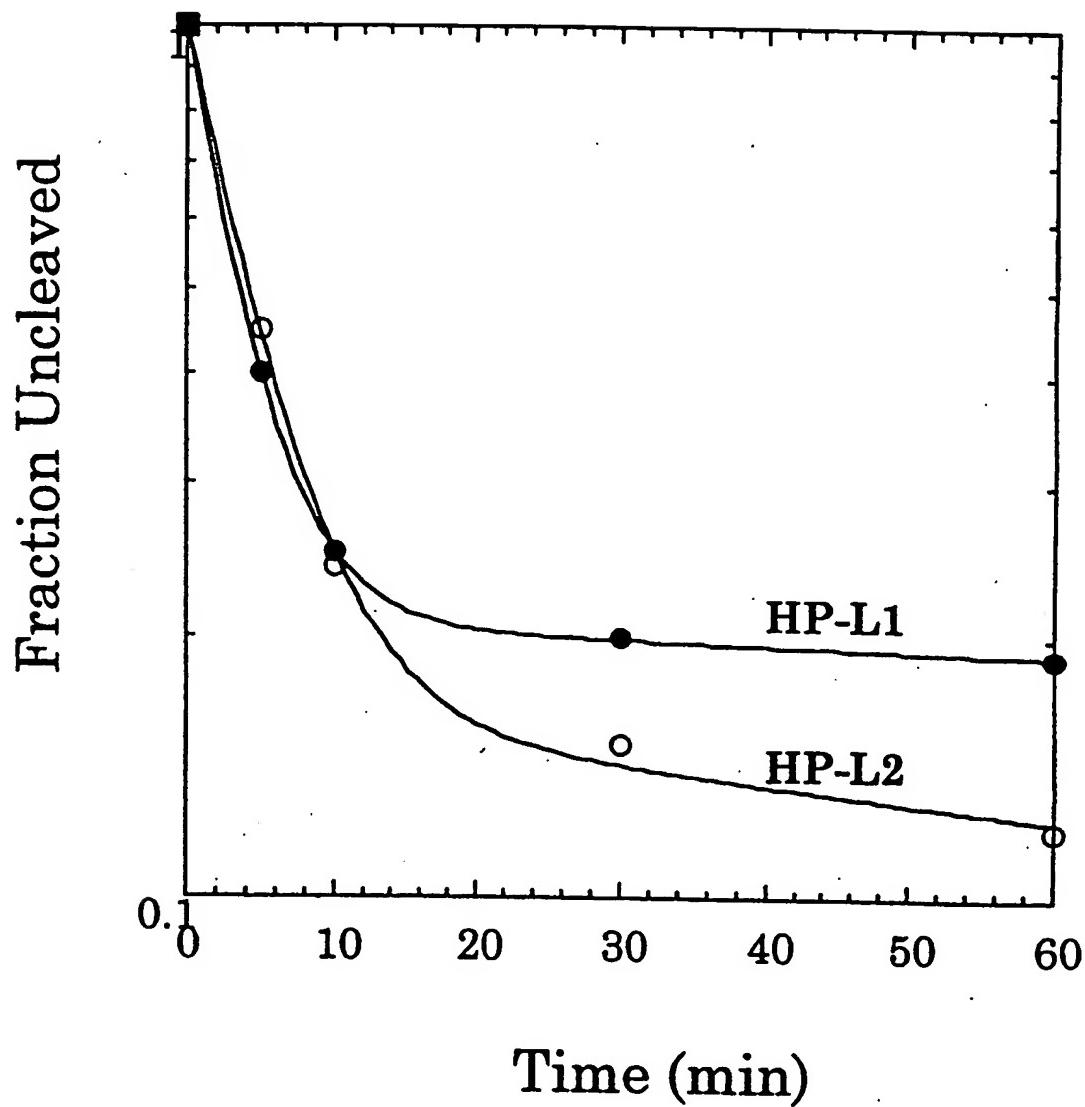


FIG. 70.

FIG. 71a.

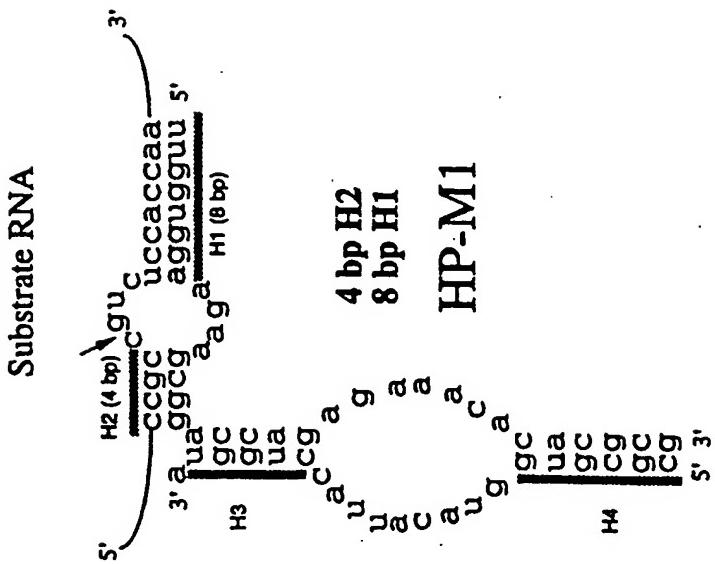
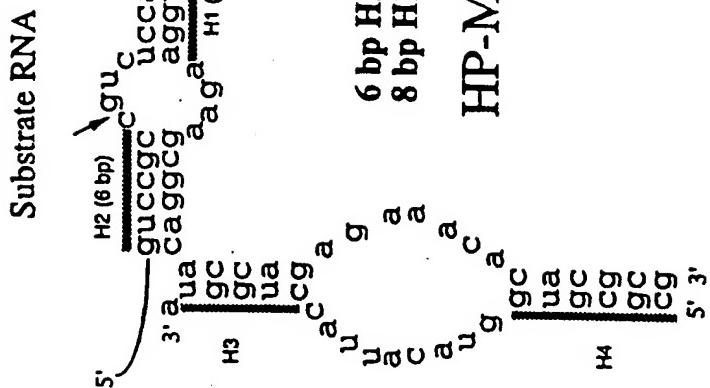


FIG. 71b.



SUBSTITUTE SHEET (RULE 26)

68/103

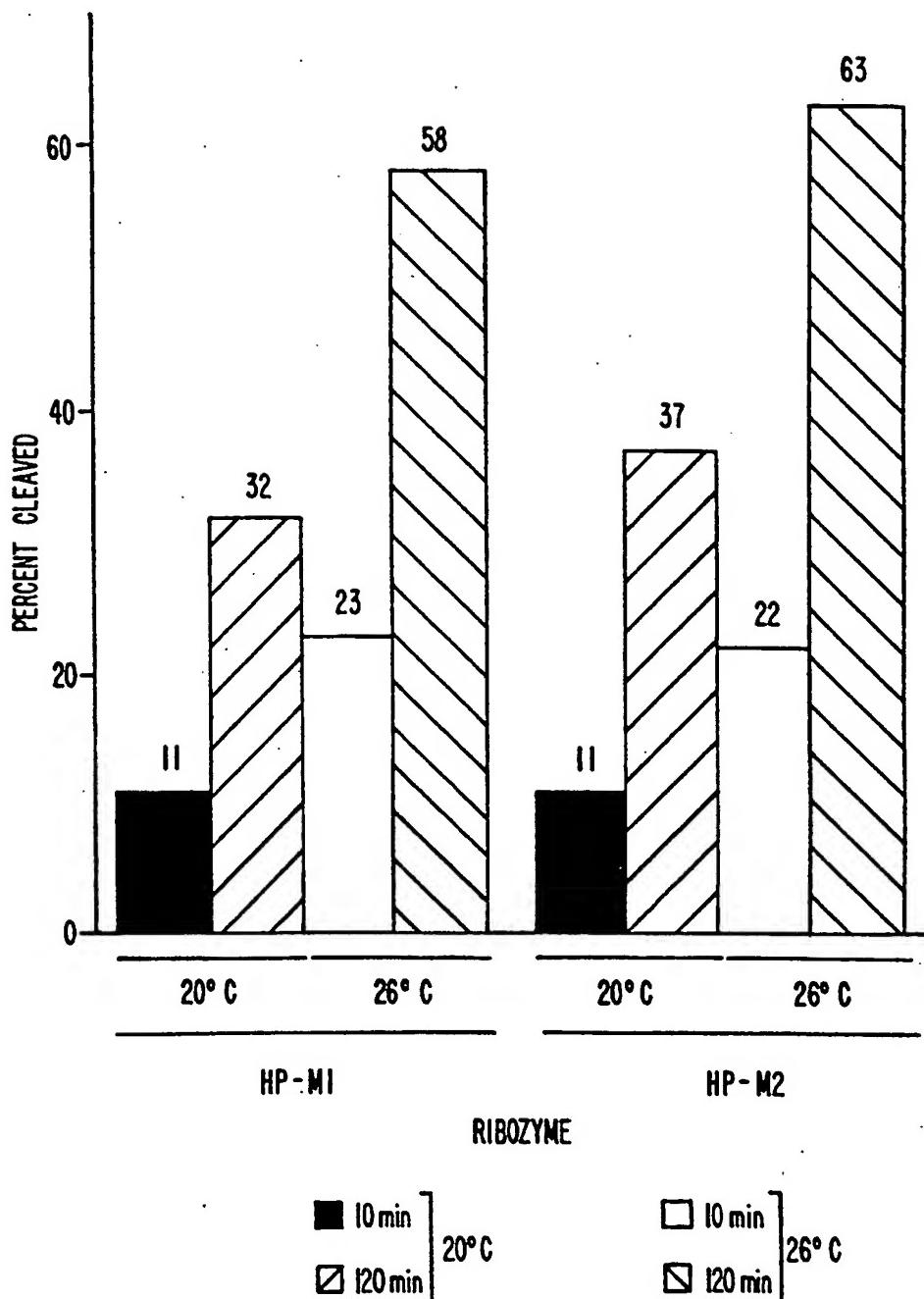


FIG. 72.

69/103

SUBSTRATE RNA

A

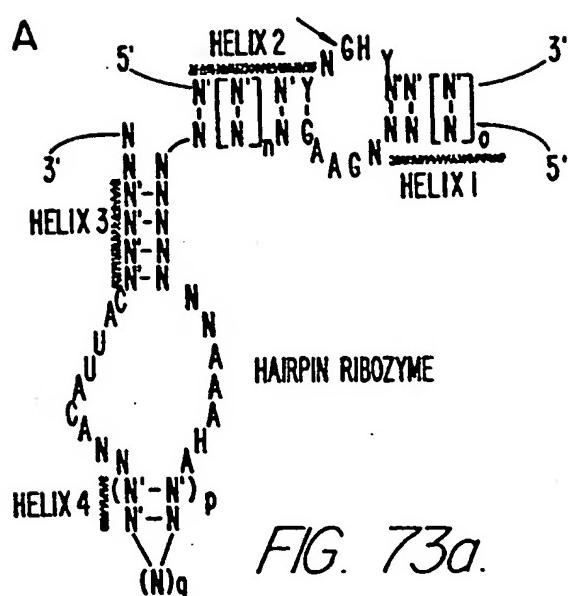


FIG. 73a.

SUBSTRATE RNA

C

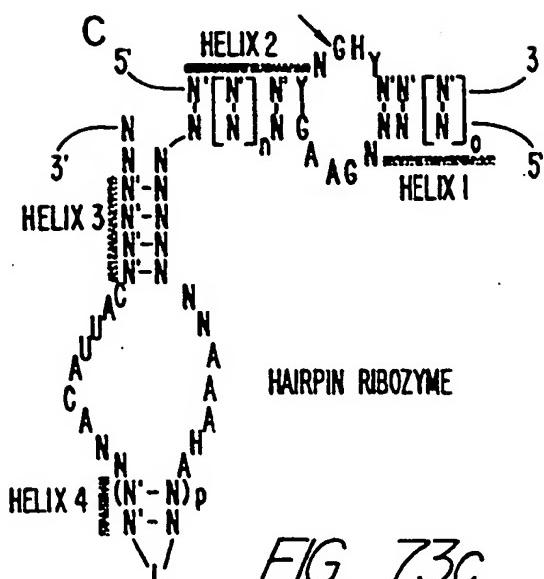


FIG. 73c.

SUBSTRATE RNA

B

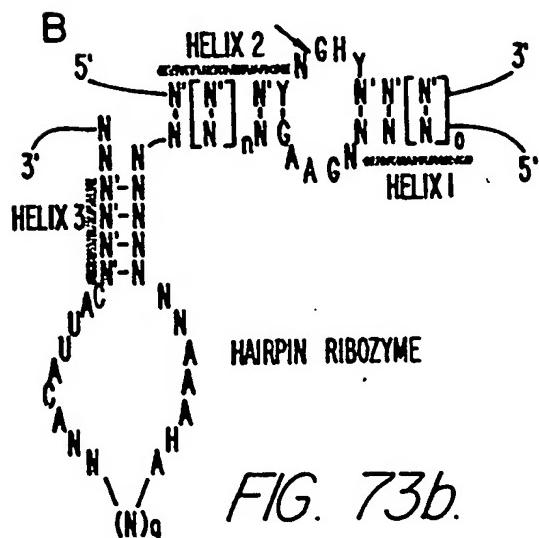


FIG. 73b.

SUBSTRATE RNA

D

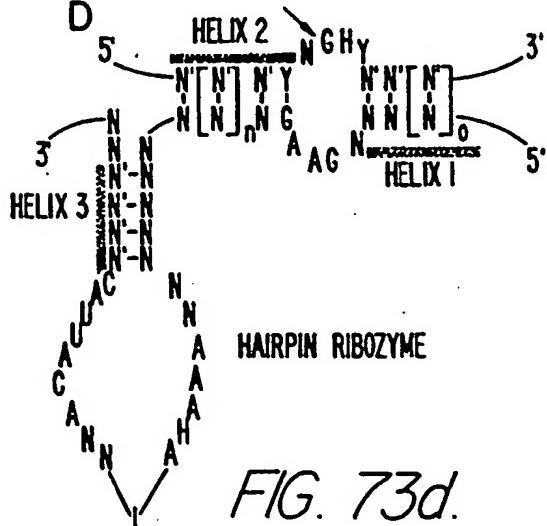


FIG. 73d.

FIG. 74a.

A

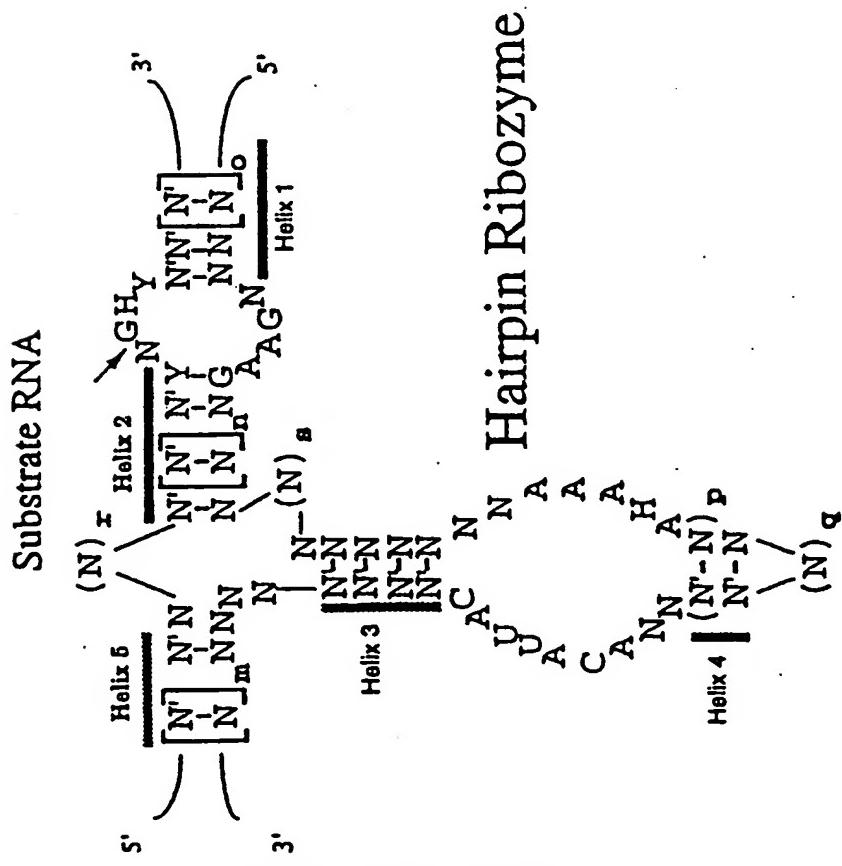
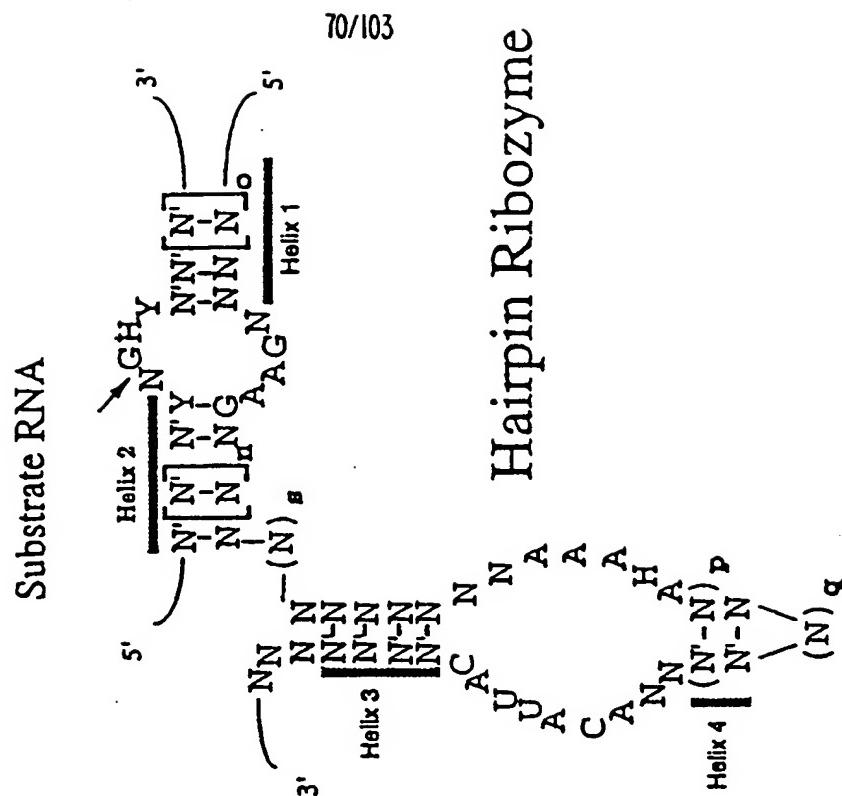


FIG. 74b.

B



71/103

FIG. 75a.

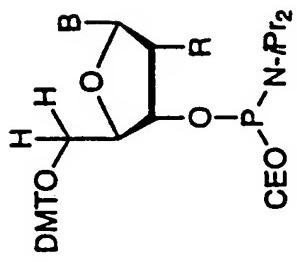


FIG. 75b.

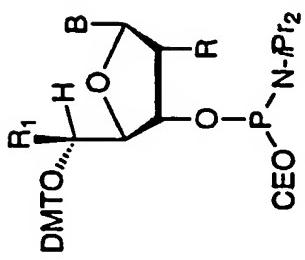


FIG. 75c.

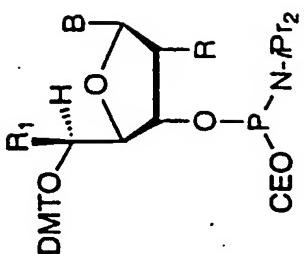
**D-Ribose Family****D-Allose Family****L-Talose Family**

FIG. 75d.

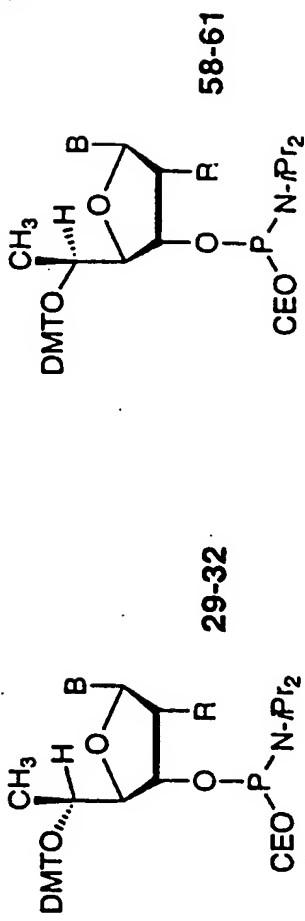
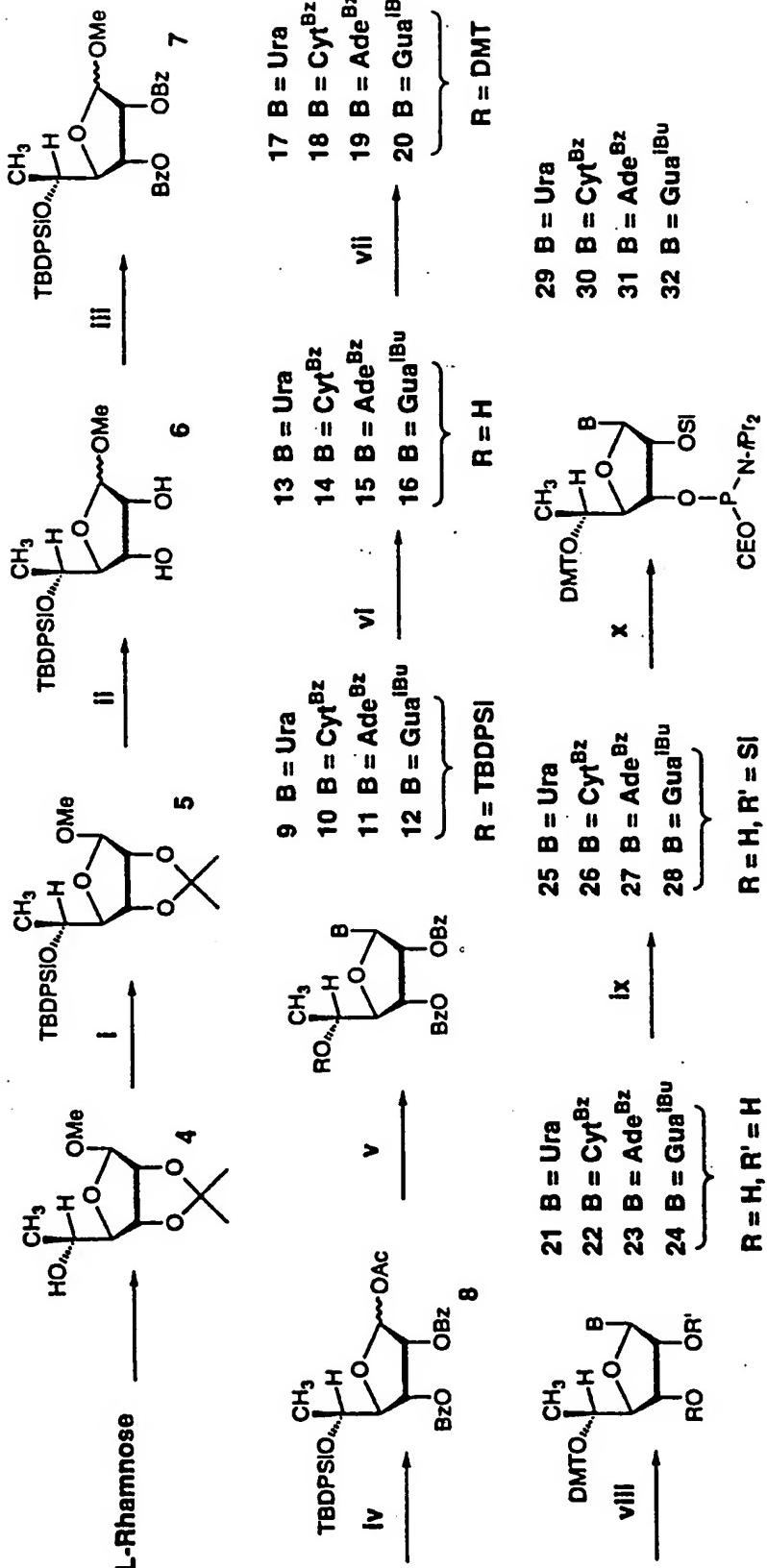
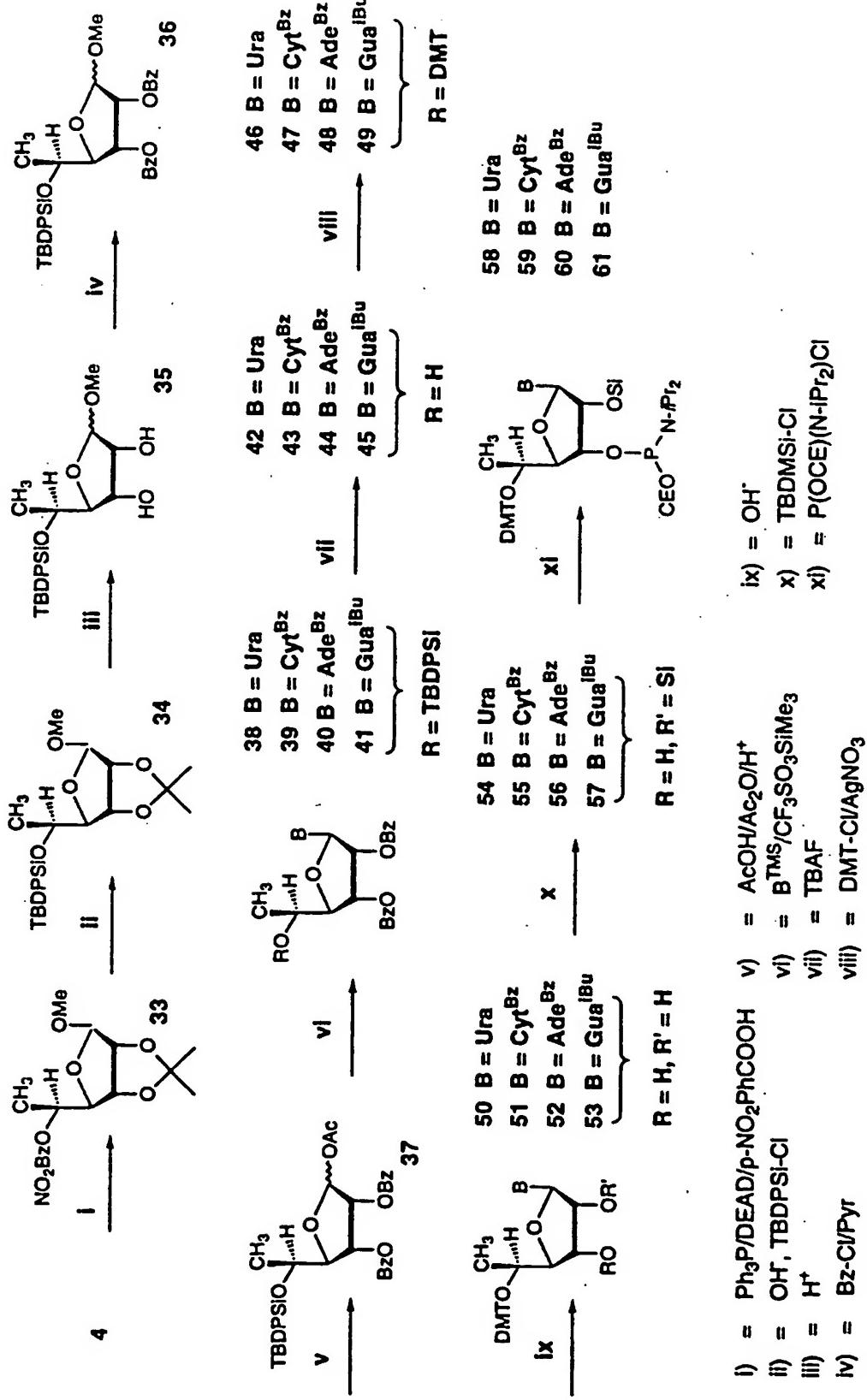


FIG. 75e. L-Talose

72/103

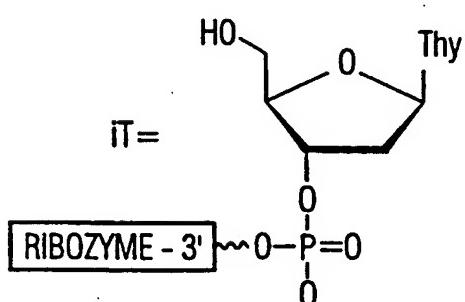
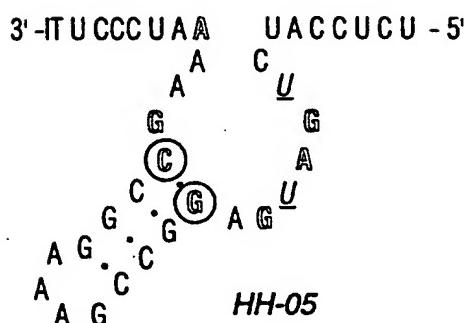
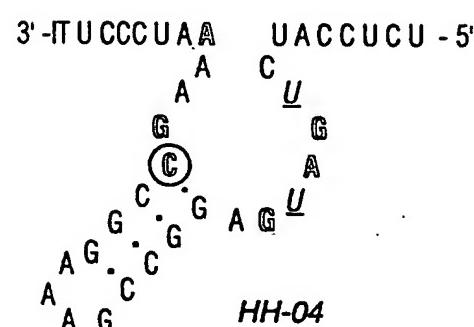
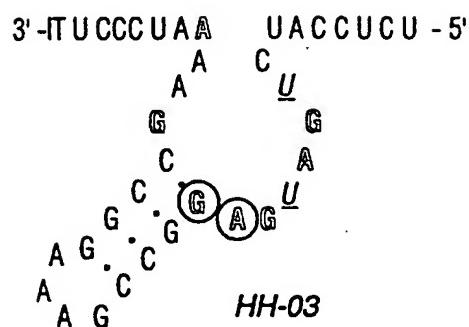
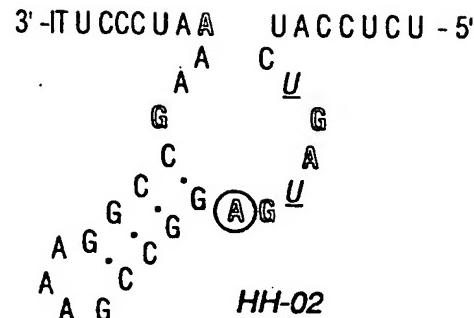
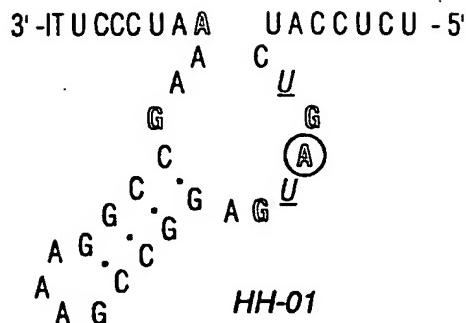


73/103



74/103

FIG. 78.



$N=2'$ -O-Me	$N=RIBO$
$U=2'$ -NH ₂ U	(N)=TALO

WHERE THE ALPHABET "N" REPRESENTS A NUCLEOTIDE, A, U, G, OR C
SUBSTITUTE SHEET (RULE 26)

75/103

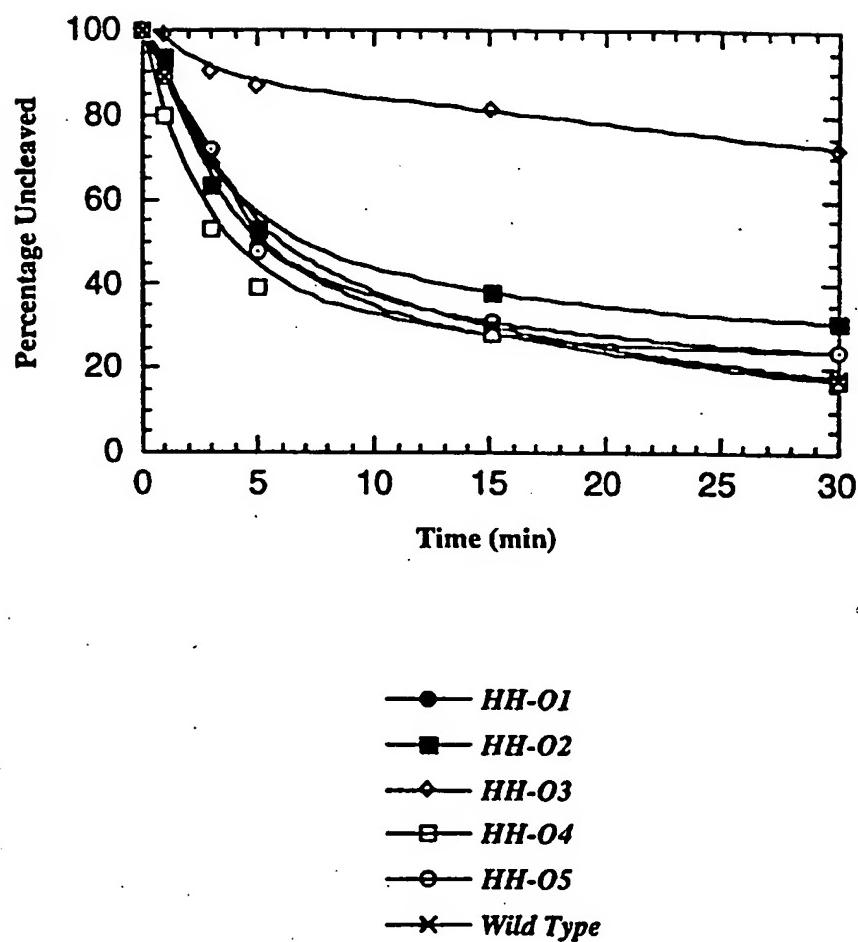
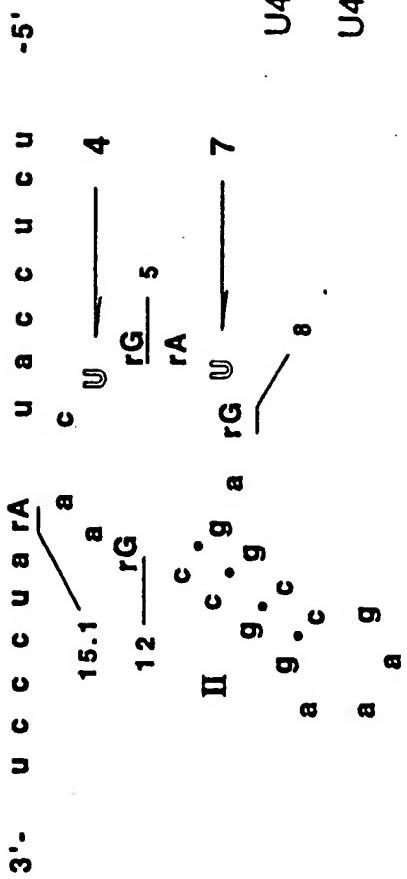


FIG. 79.

76/103

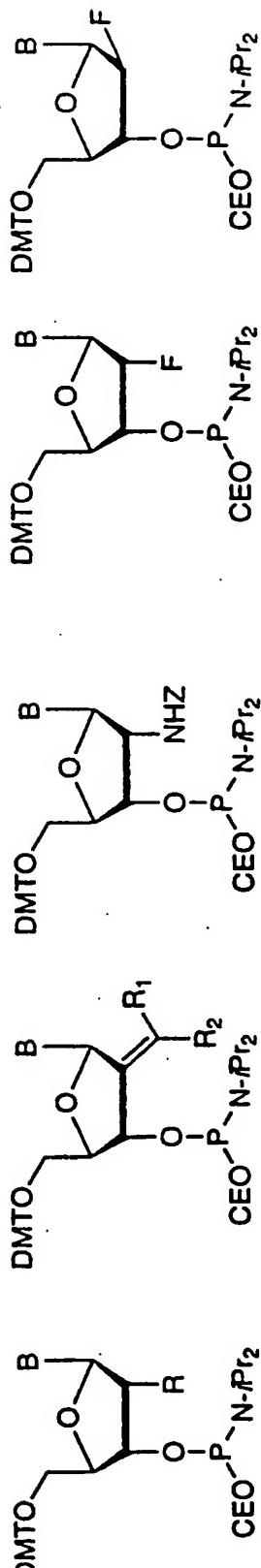
Table 1 Entries

U4 & U7 = 2'-C-Allyl-U	12-14
U4 & U7 = 2'-F-ribo-U	9-11
U4 & U7 = 2'=CH ₂ -U	3-5
U4 & U7 = 2'=CF ₂ -U	6-8
U4 & U7 = 2'-dU	21-22
U4 & U7 = 2'-F-ara-U	15-17
U4 & U7 = 2'-NH ₂ -U	18-20
U4 & U7 = 2'-O-Me-ribo-U	2

Lower case = 2'-O-Me
ribonucleotide
rN = ribonucleotide

FIG. 80.

77/103



10 & 12 *FIG. 8/f.* DMTO-*B*-O-P(=O)(OR)-C=C

17, 22 & 31 *FIG. 8/g.* DMTO-*B*-O-P(=O)(OR)-C(H)=C(H)F

18, 26 & 32 *FIG. 8/h.* DMTO-*B*-O-P(=O)(OR)-C(H)=C(H)OR₃

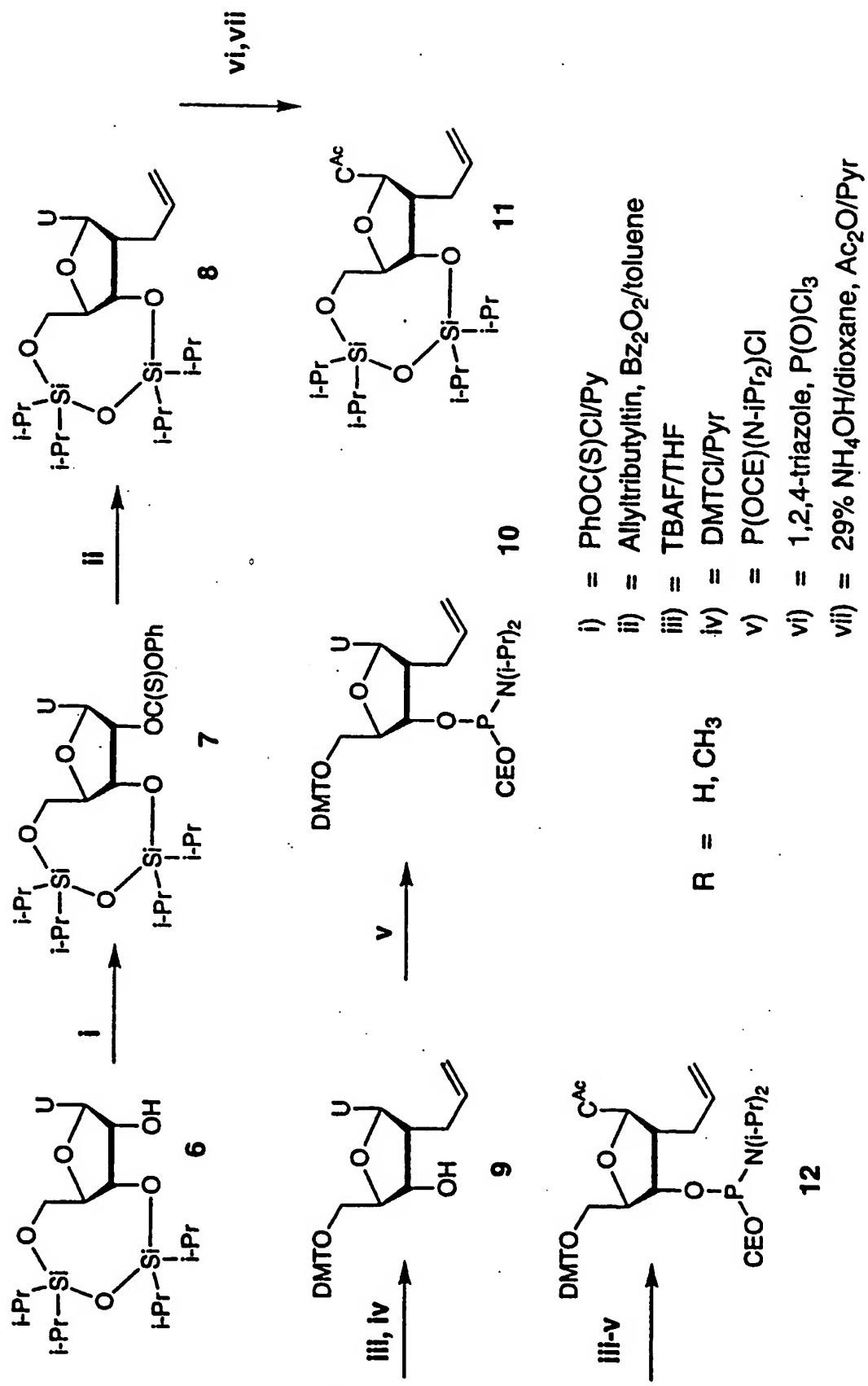
36 *FIG. 8/i.* DMTO-*B*-O-P(=O)(OR)-C(H)=C(H)X

38 *FIG. 8/j.* DMTO-*B*-O-P(=O)(OR)-C(H)=C(H)C(=O)H

SUBSTITUTE SHEET (RULE 26)

B = Protected A, C, G, U, T, 2AP, I, DiAP, P etc.

78/103



79/103

FIG. 83.

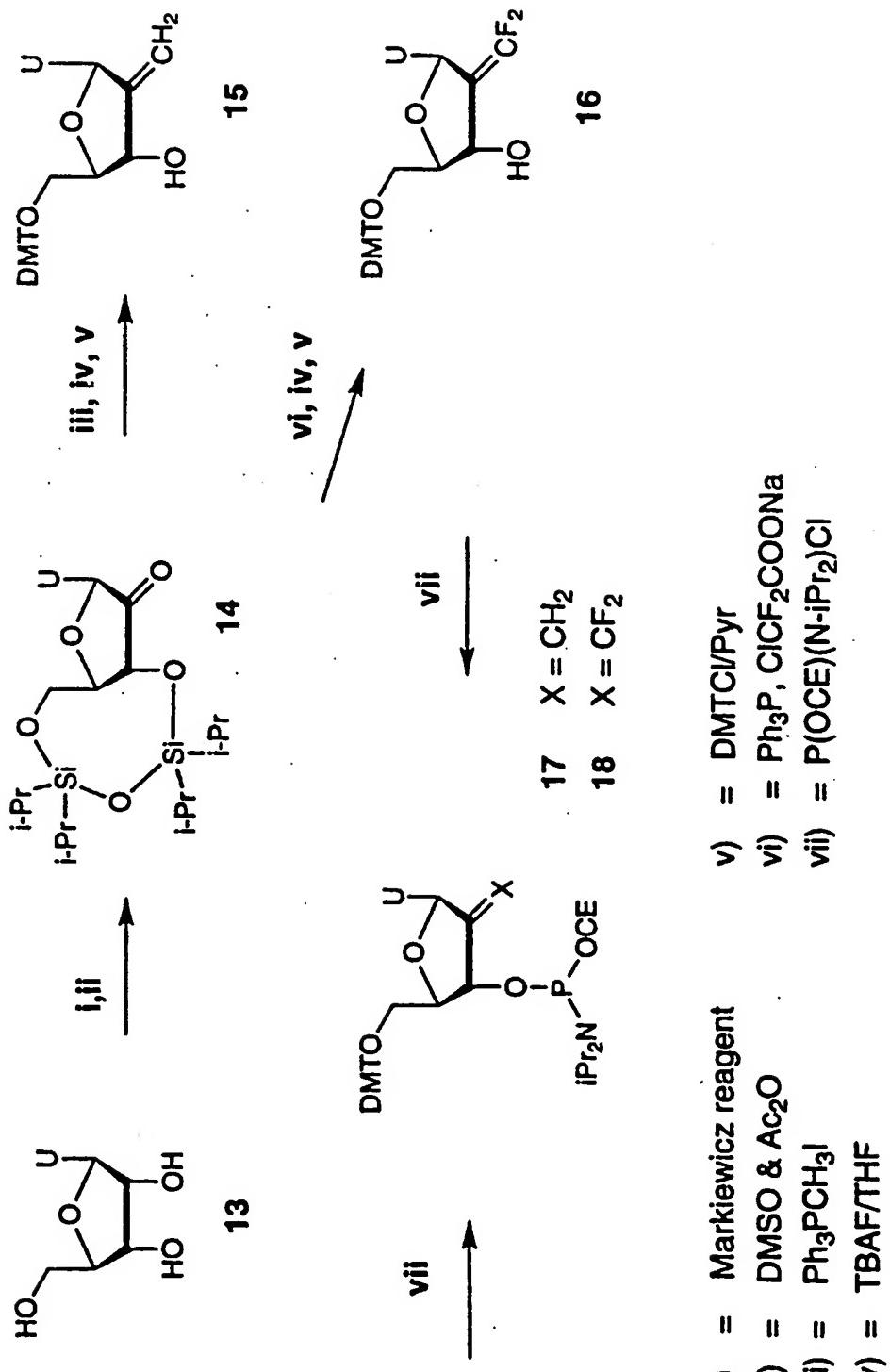
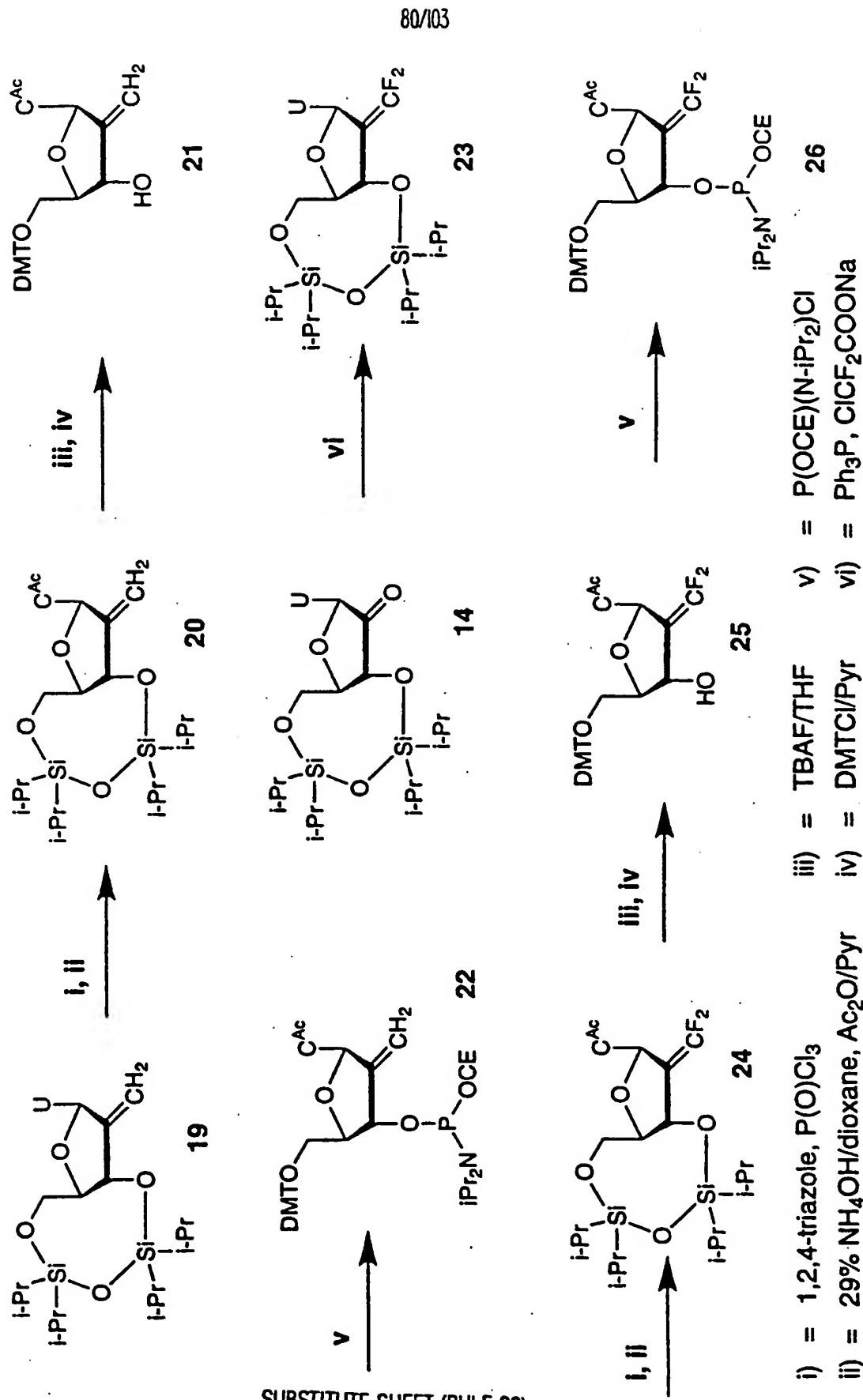


FIG. 84.



81/103

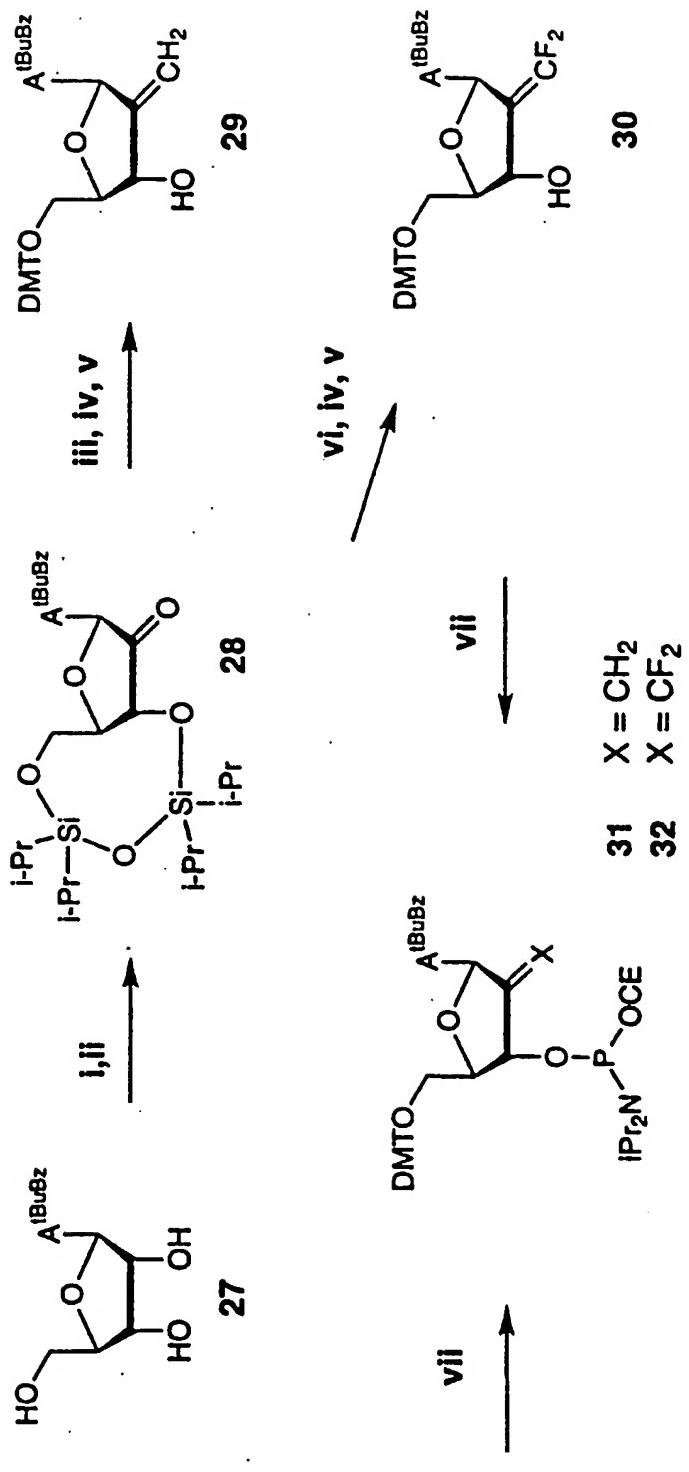
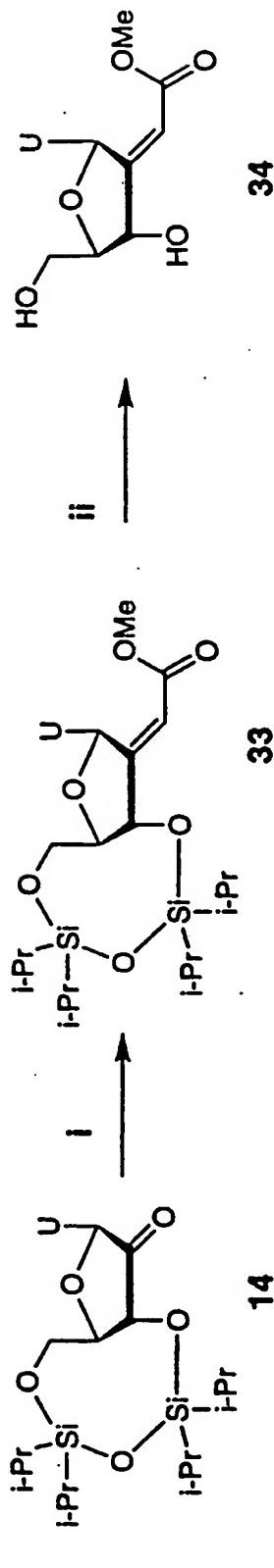


FIG. 85.

82/103



i) = $\text{Ph}_3\text{PC}=\text{CHC}(\text{O})\text{OCH}_3\bullet\text{OAc}$
 ii) = $\text{NEt}_3\bullet 3 \text{ HF}$
 iii) = DMTCl/Pyr
 iv) = $\text{P(OCE)}(\text{N-iPr}_2)\text{Cl}$
 v) = MeOH/NaOH

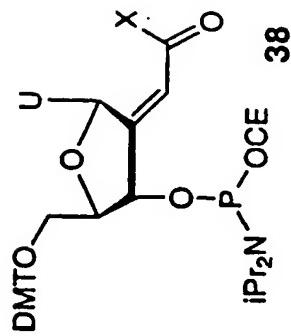
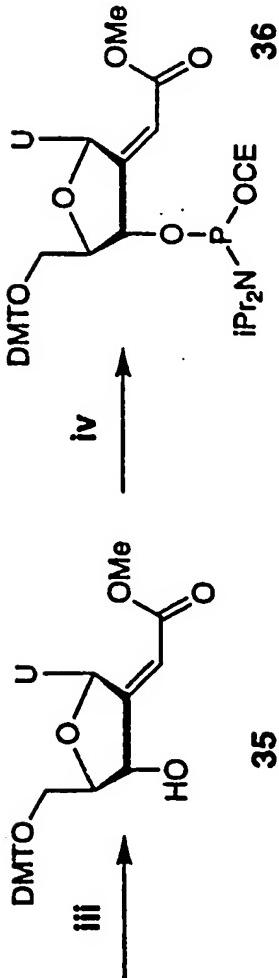
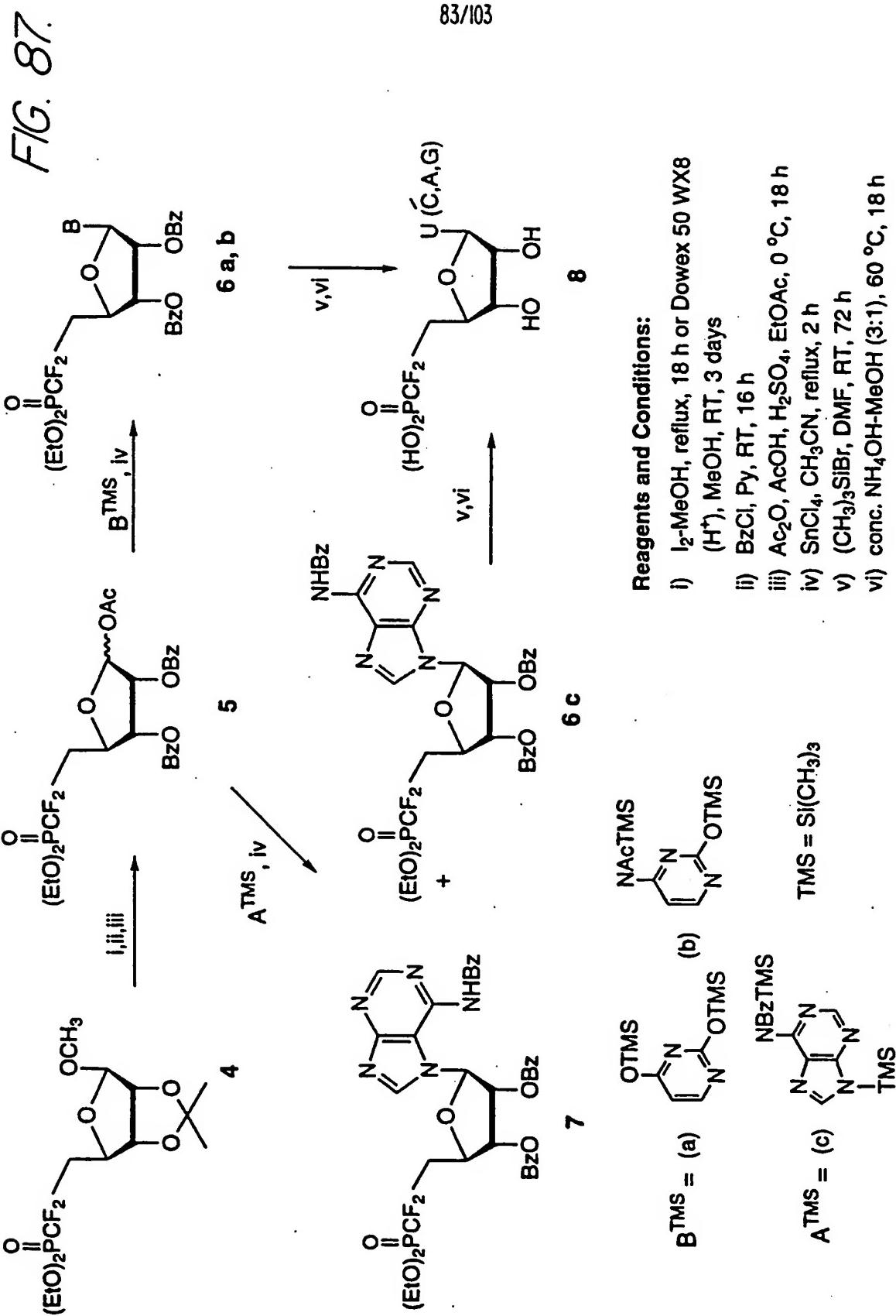


FIG. 86.



84/103

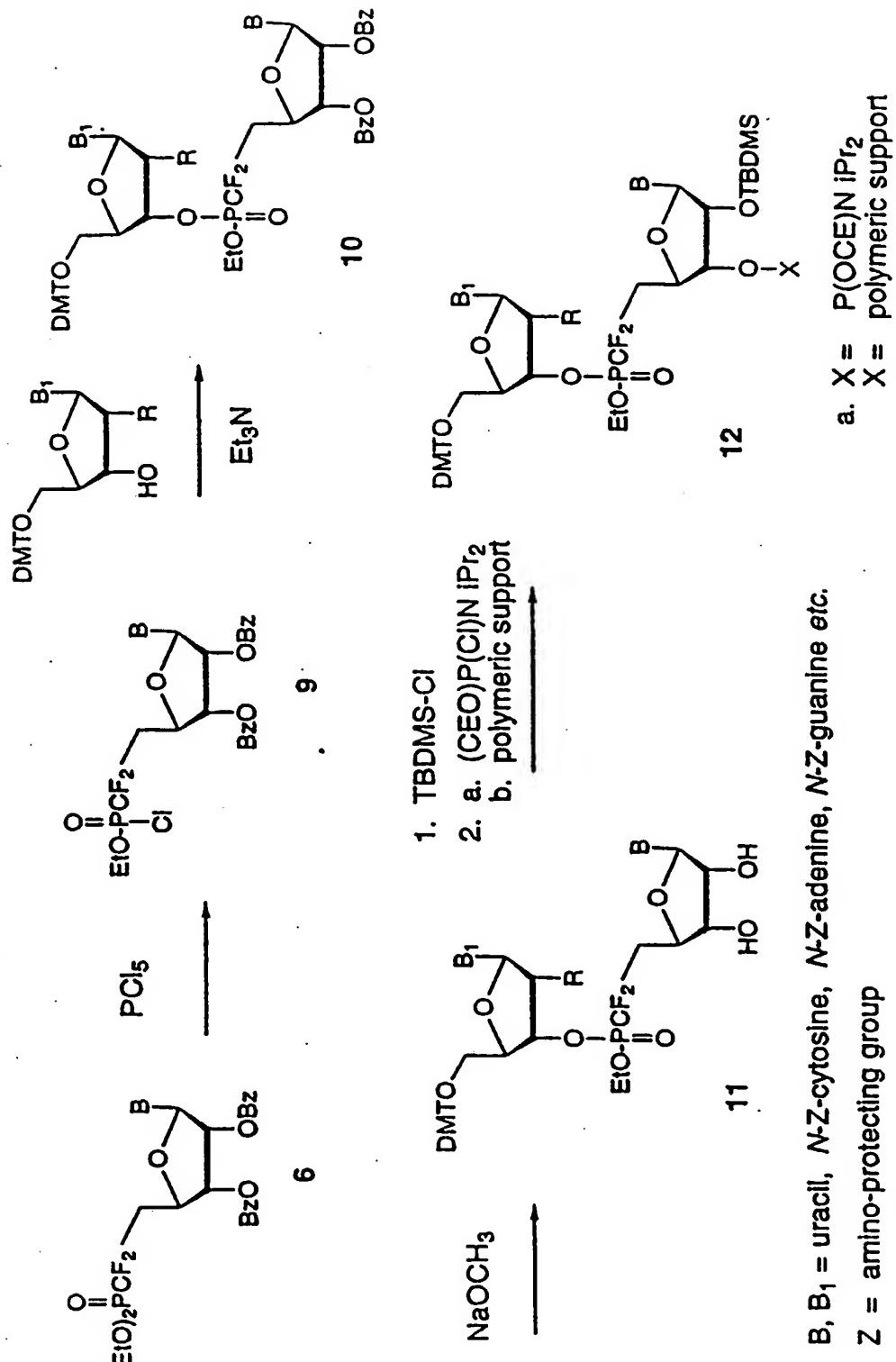
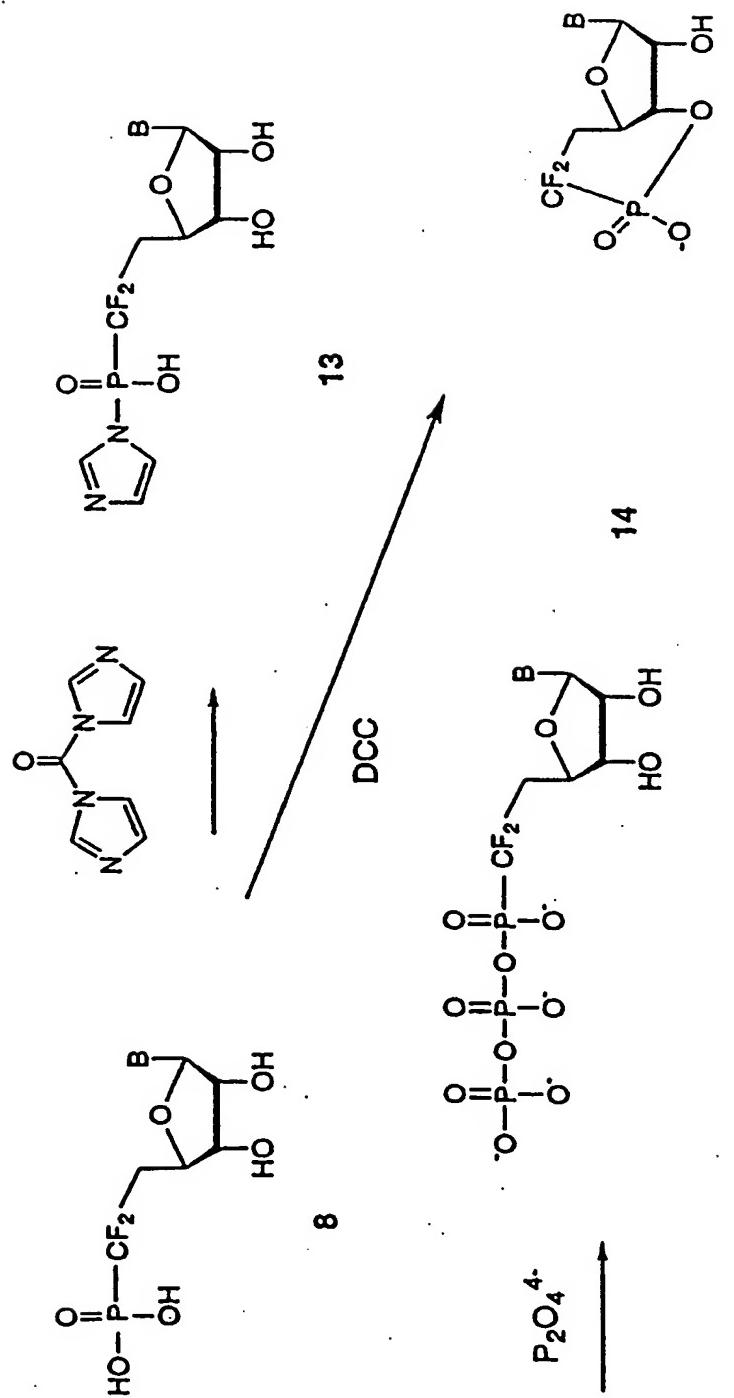


FIG. 88.

85/103

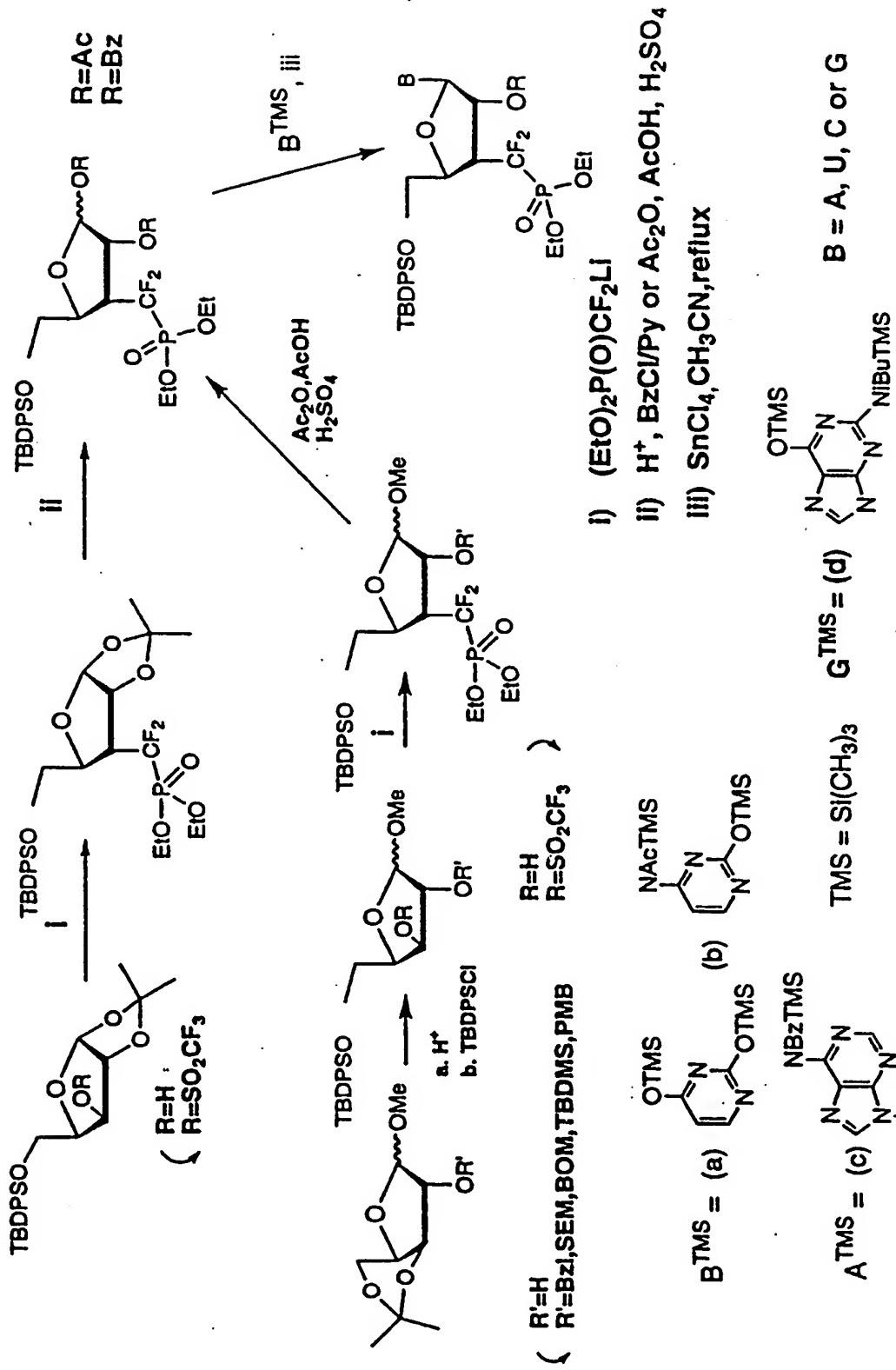


B = uracil, N-Z-cytosine, N-Z-adenine, N-Z-guanine etc.

Z = amino-protecting group

FIG. 89.

86/103



87/103

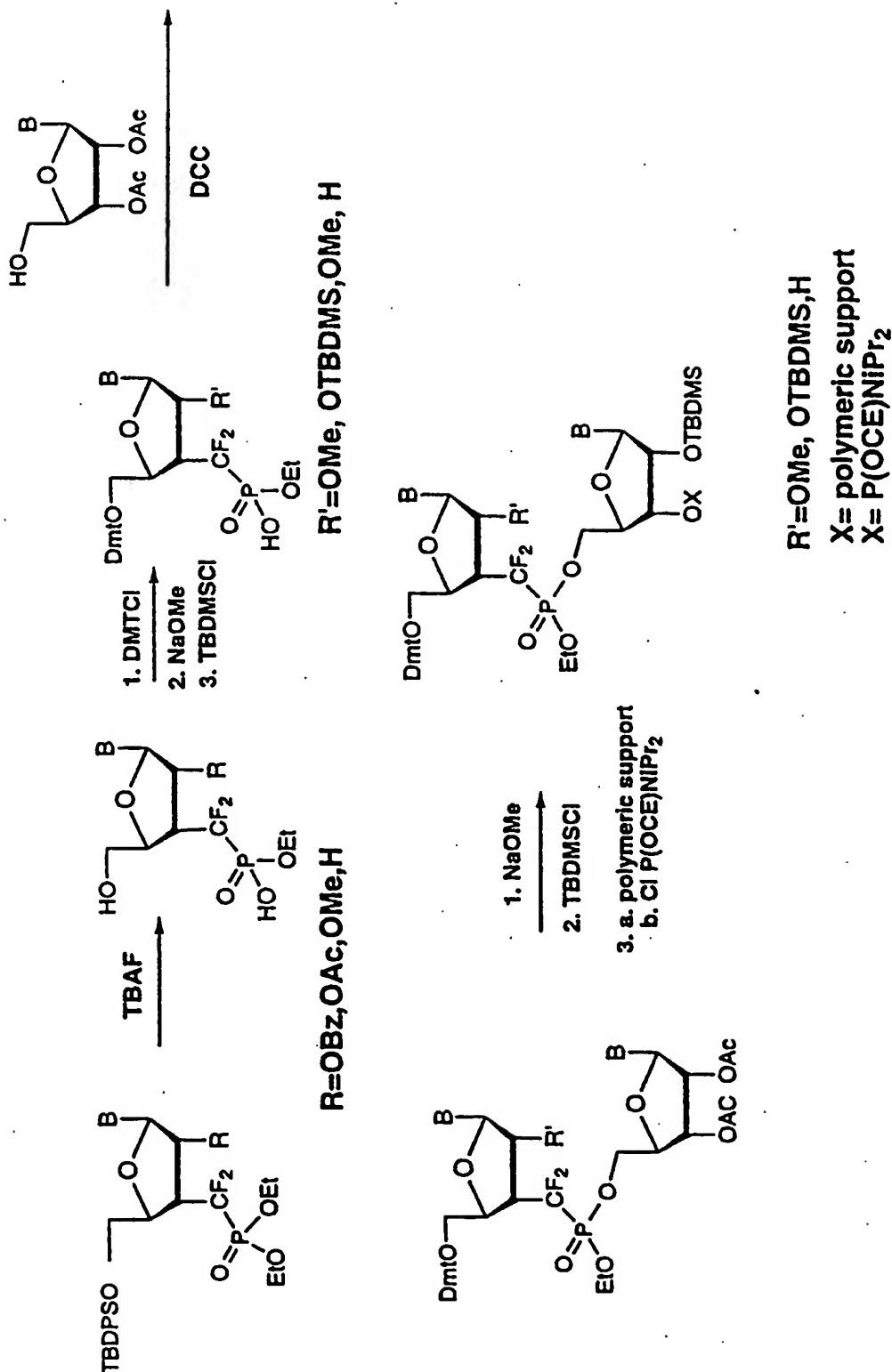


FIG. 9/

88/103

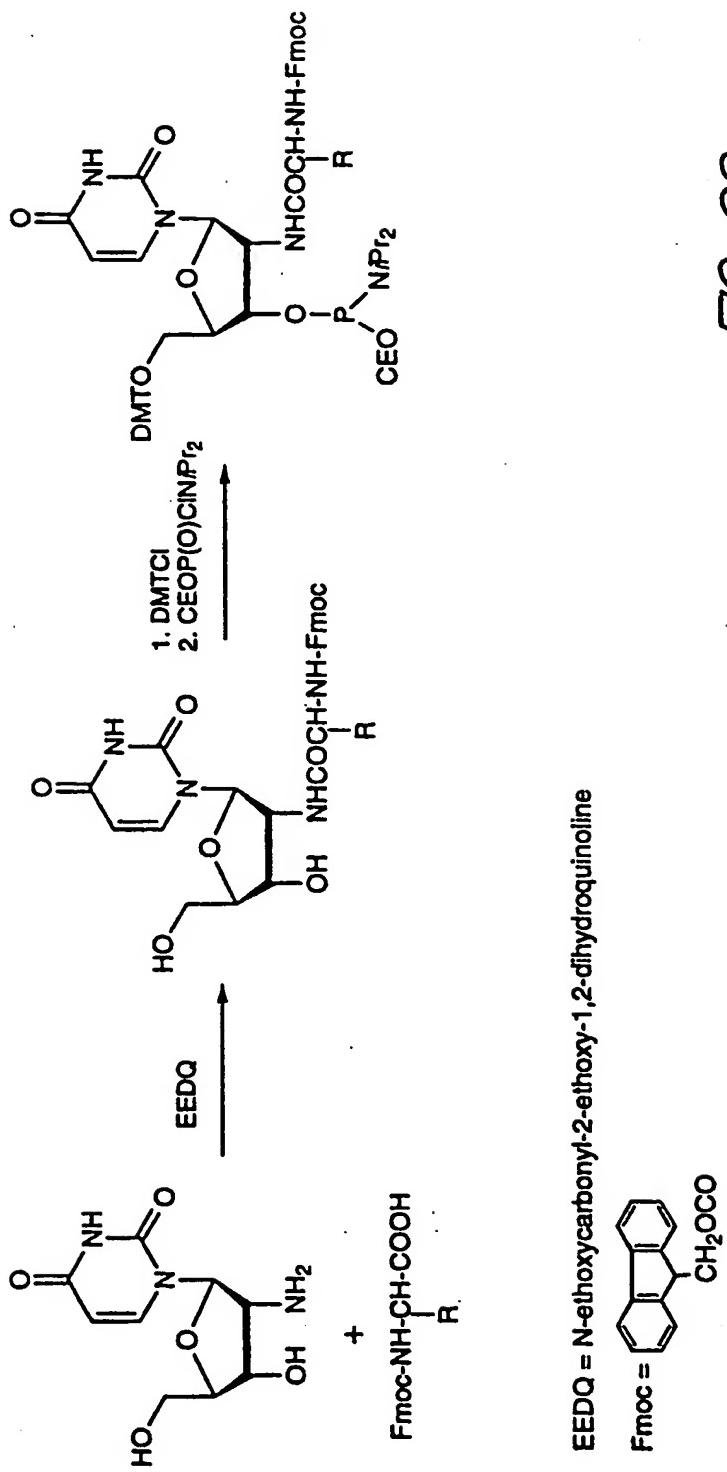


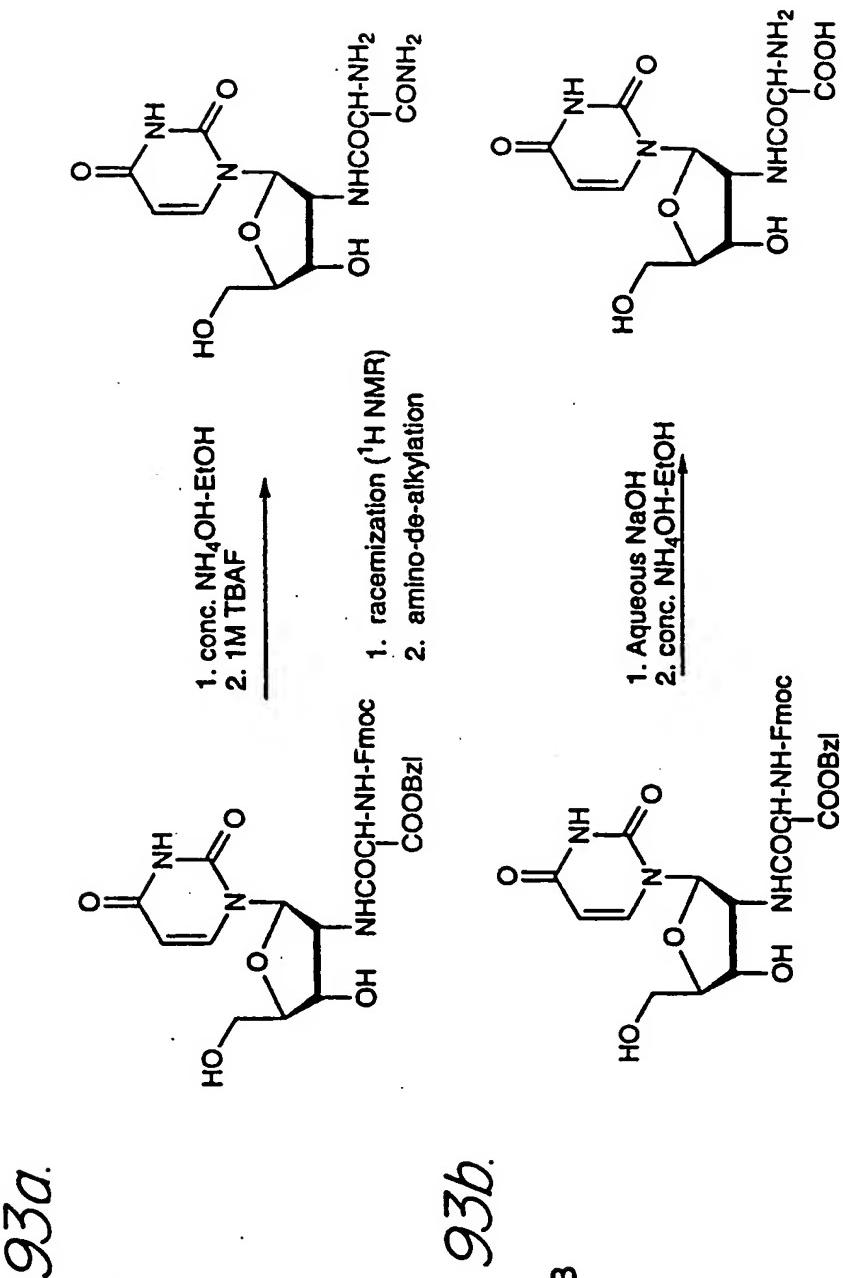
FIG. 92.

SUBSTITUTE SHEET (RULE 26)
 EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
 Fmoc =

 R = CH₃, CH₂-phenyl (ala), (phe)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBz (lys)
 CBZ =

 BzI =

89/103



90/103

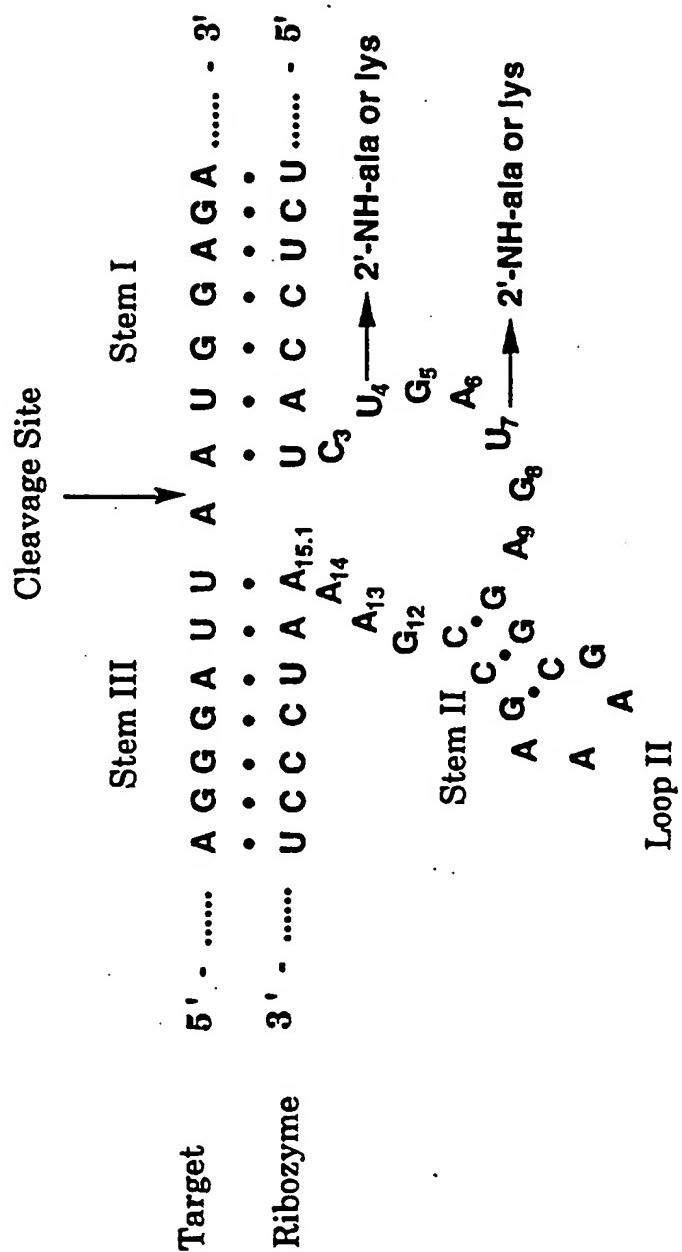
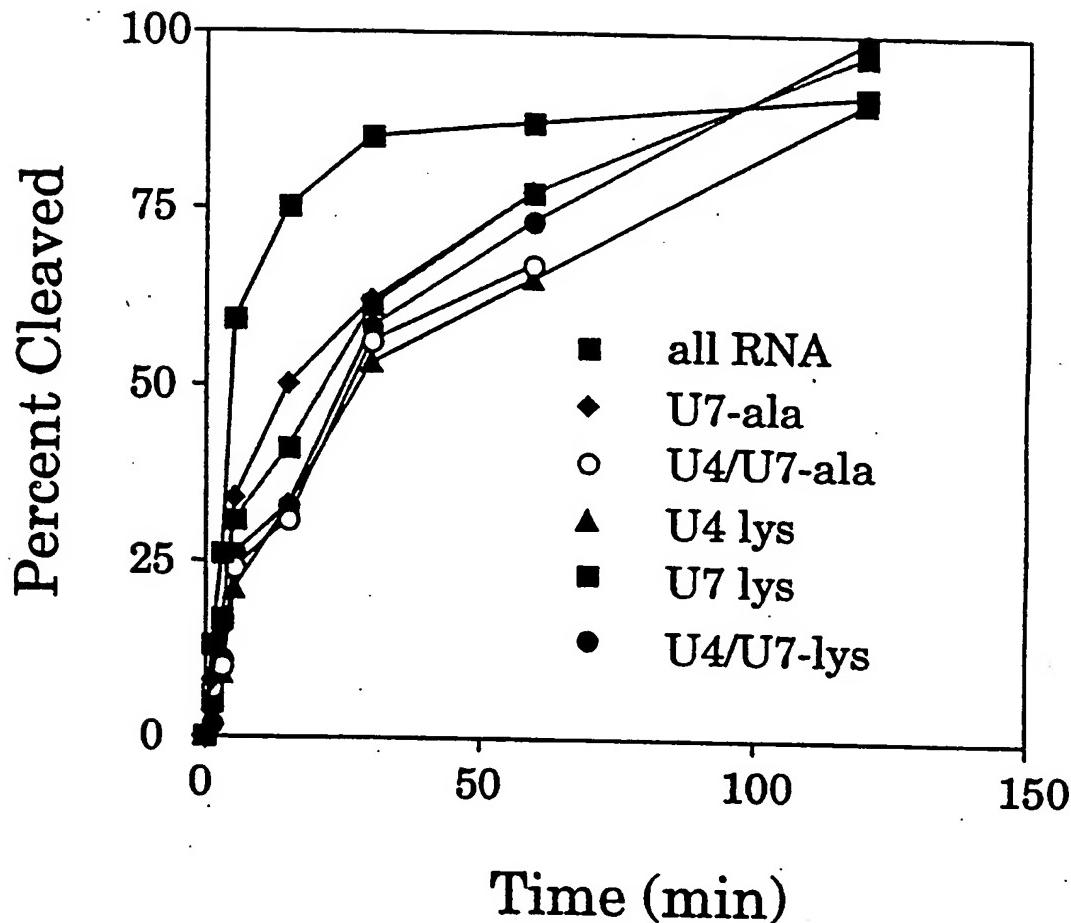


FIG. 94.

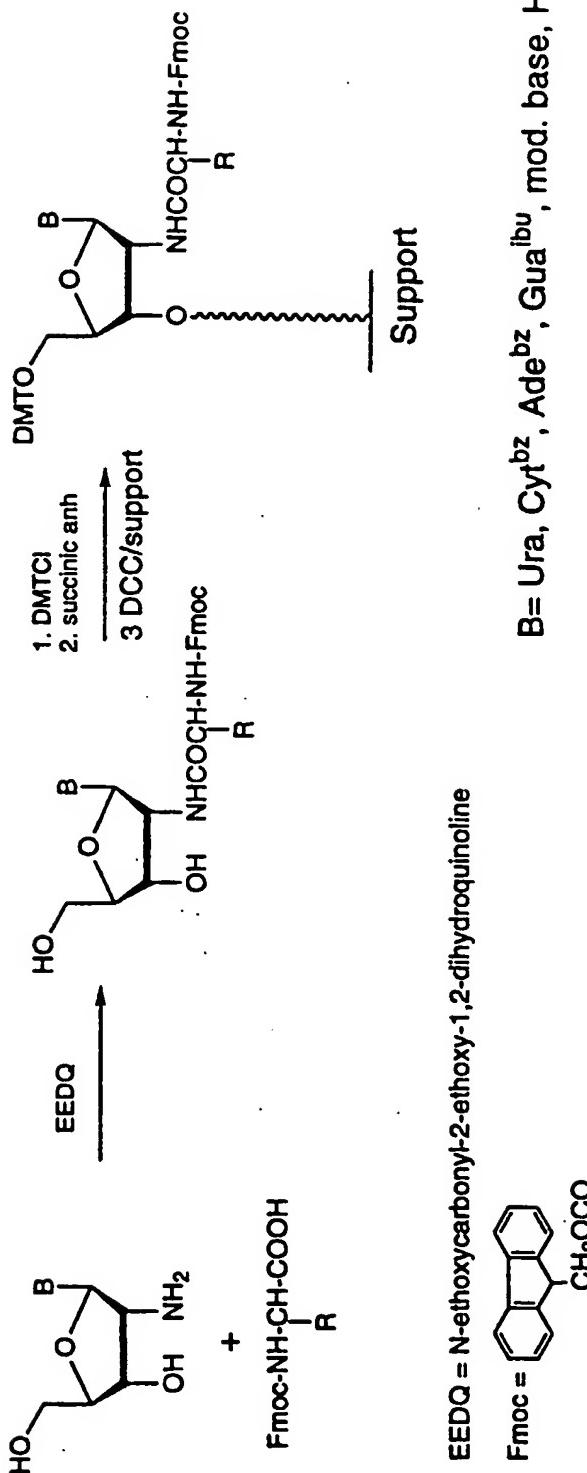
91/103



[Ribozyme] = 40 nM [Substrate] = ~1nM

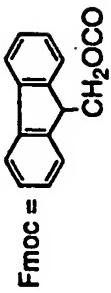
FIG. 95.

92/103



B= Ura, Cyt^{bz}, Ade^{bz}, Gua^{bz}, mod. base, H

EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline



R = CH₃, CH₂-(ala), (CH₂)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBzl (lys)
 (asp)

CBZ =

Bzl =

FIG. 96.

93/103

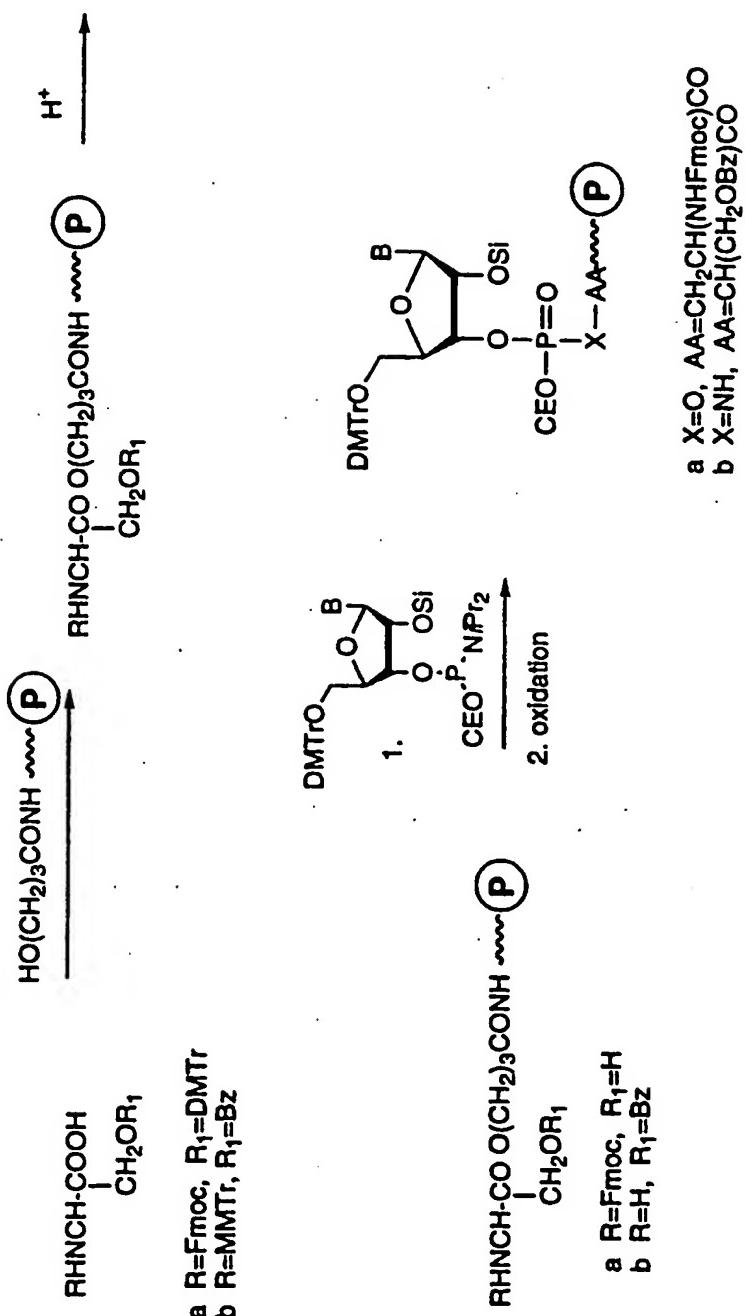
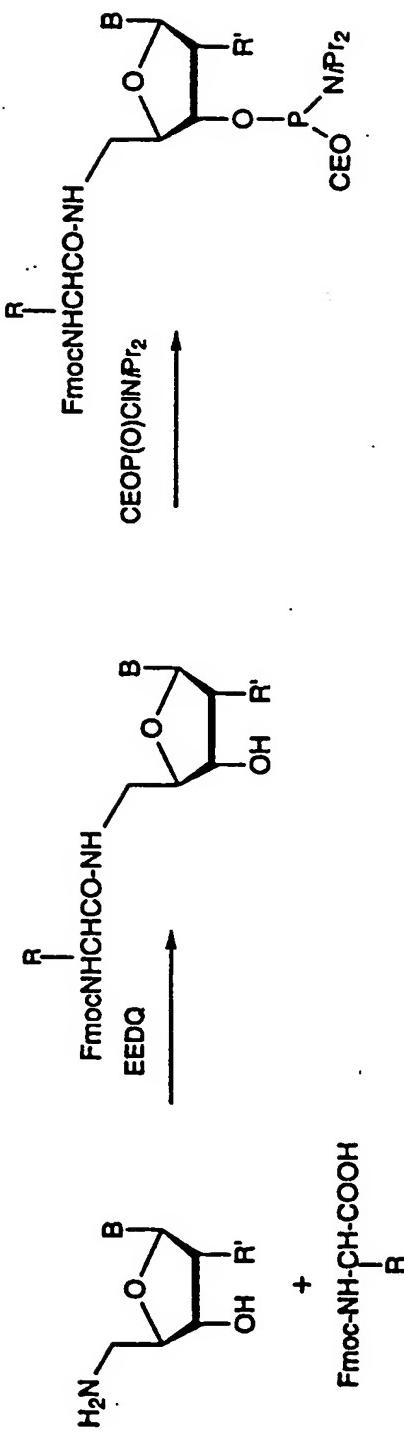
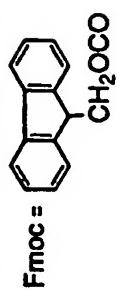


FIG. 97.

94/103



EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline



R' = H, OMe, OTBDMSi
 B = Ura, Cyt^{bz}, Ade^{bz}, Gua^{bz}, mod. base, H

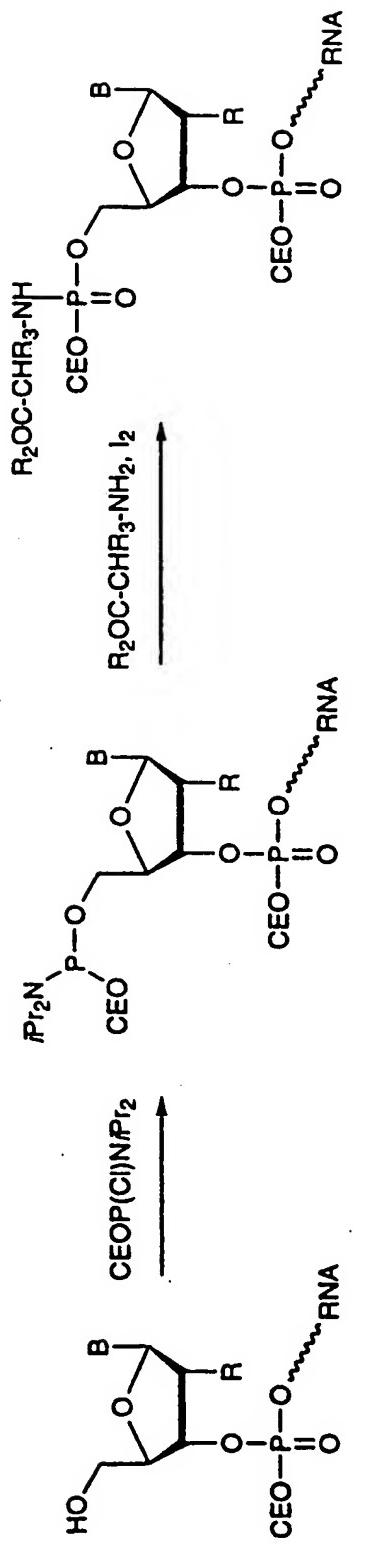
R = CH₃, CH₂-, (CH₂)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBz (lys)
 CBZ =

BzI =

FIG. 98.

95/103

FIG. 99.



B = Ura, Cy^{bz}, Ade^{bz}, Gua^{bz}, mod. base, H

R = H, OCH₃, OTBDMS, Hal, NHR₁

R₂ = OBzl, peptidyl

96/103

FIG. 100.

Reversion of mutant RNA

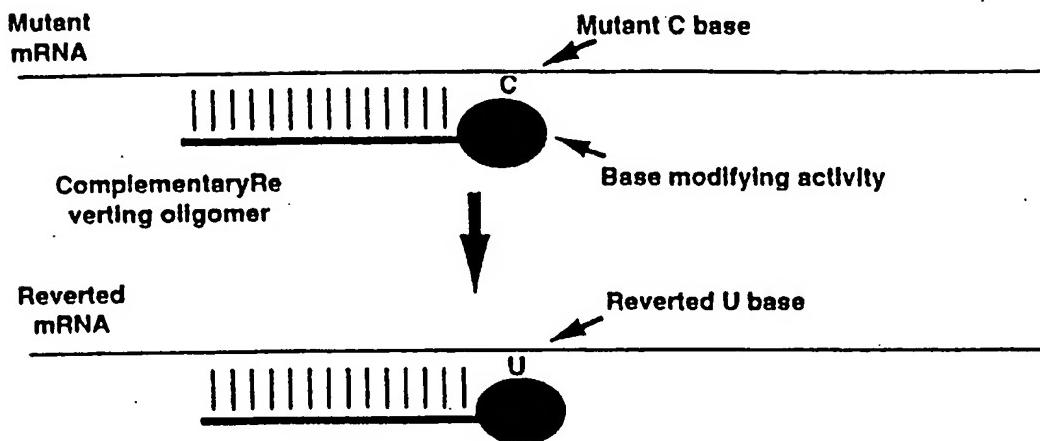
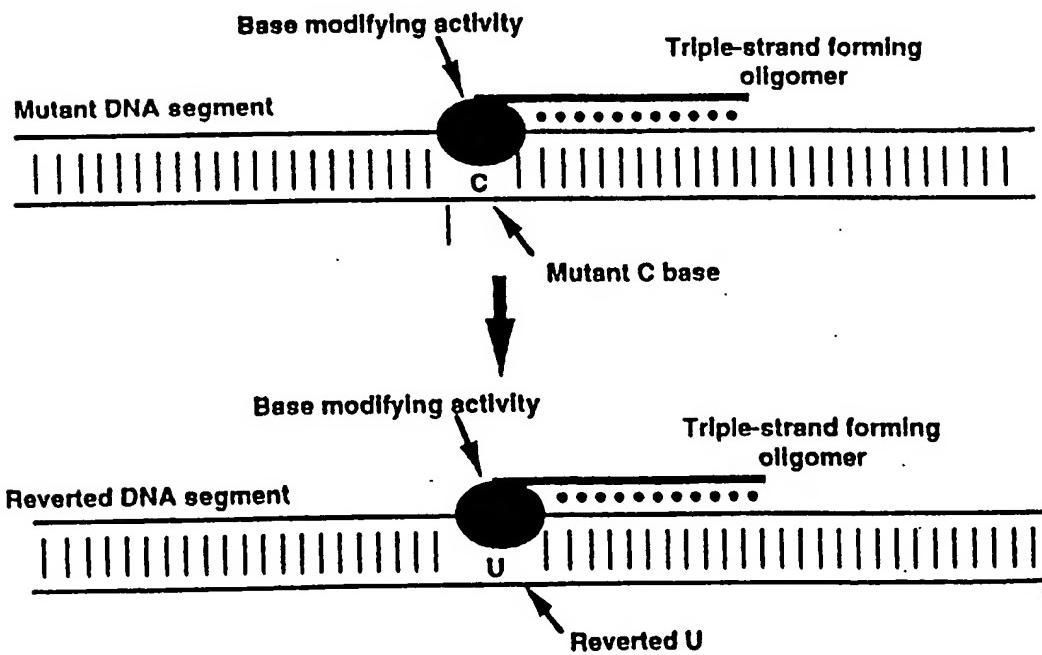


FIG. 101.

Reversion of mutant DNA



SUBSTITUTE SHEET (RULE 26)

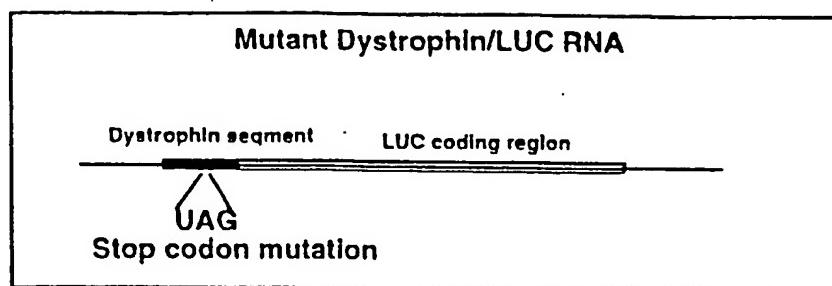


FIG. 102a.

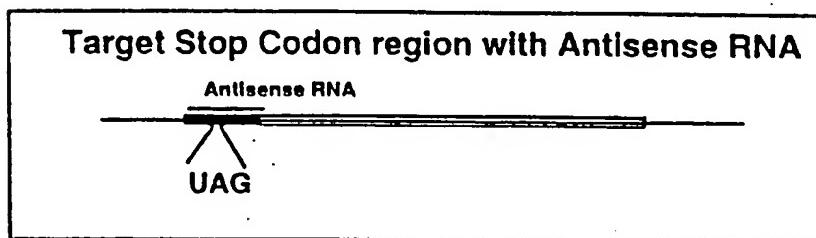


FIG. 102b.

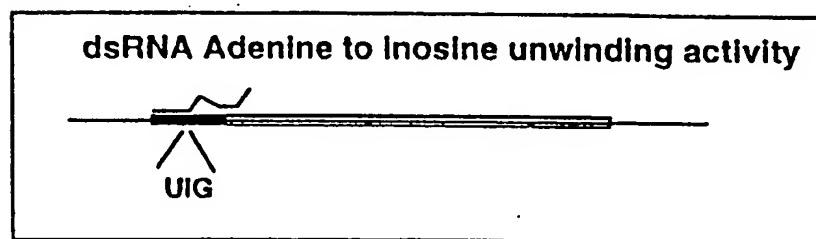


FIG. 102c.

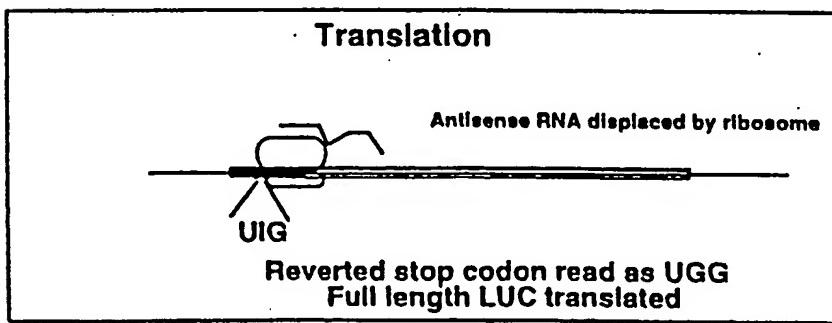
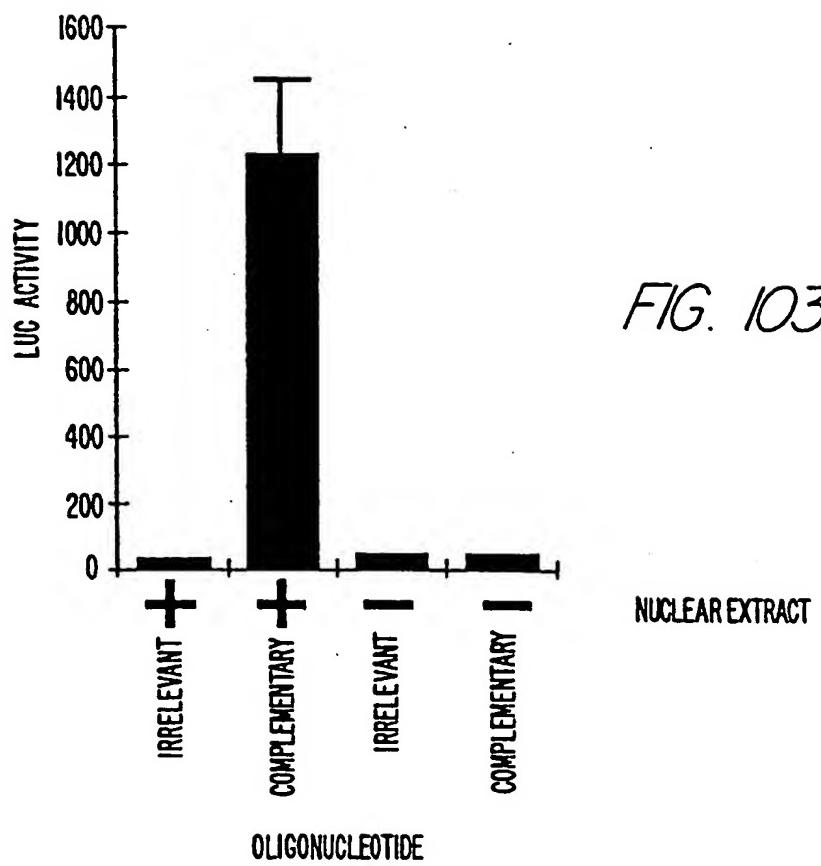


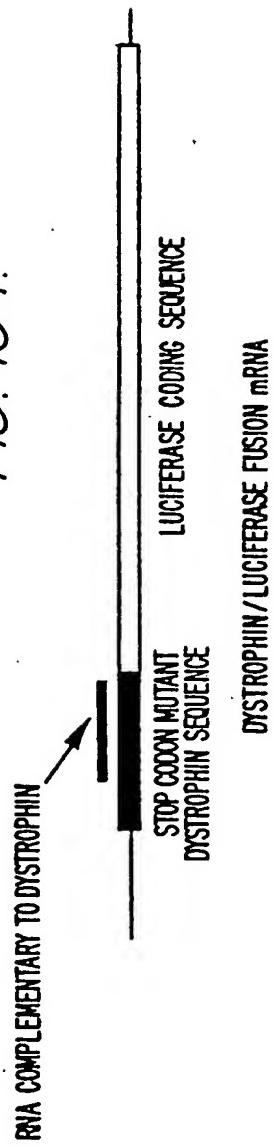
FIG. 102d.

98/103



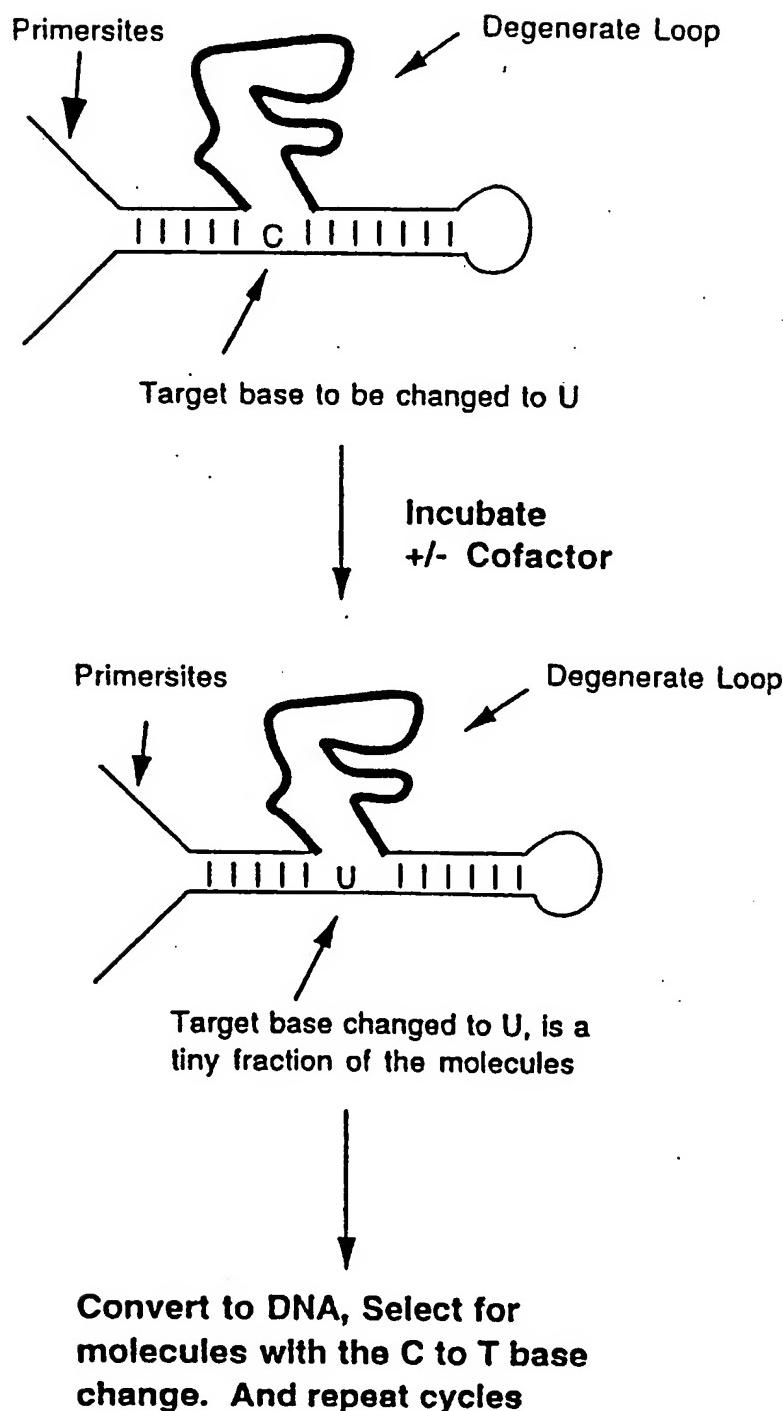
99/103

FIG. 104.



100/103

FIG. 105.



101/103

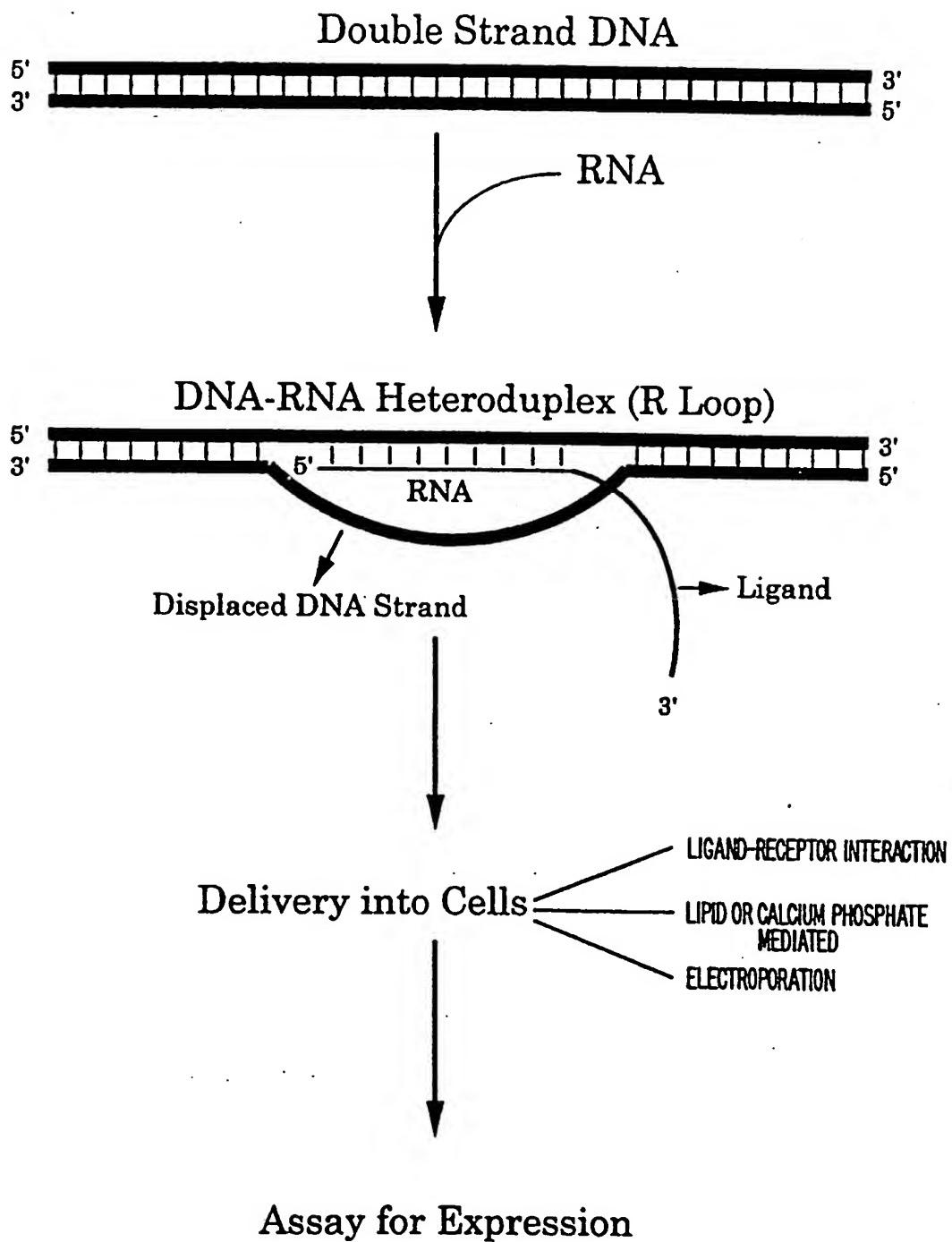
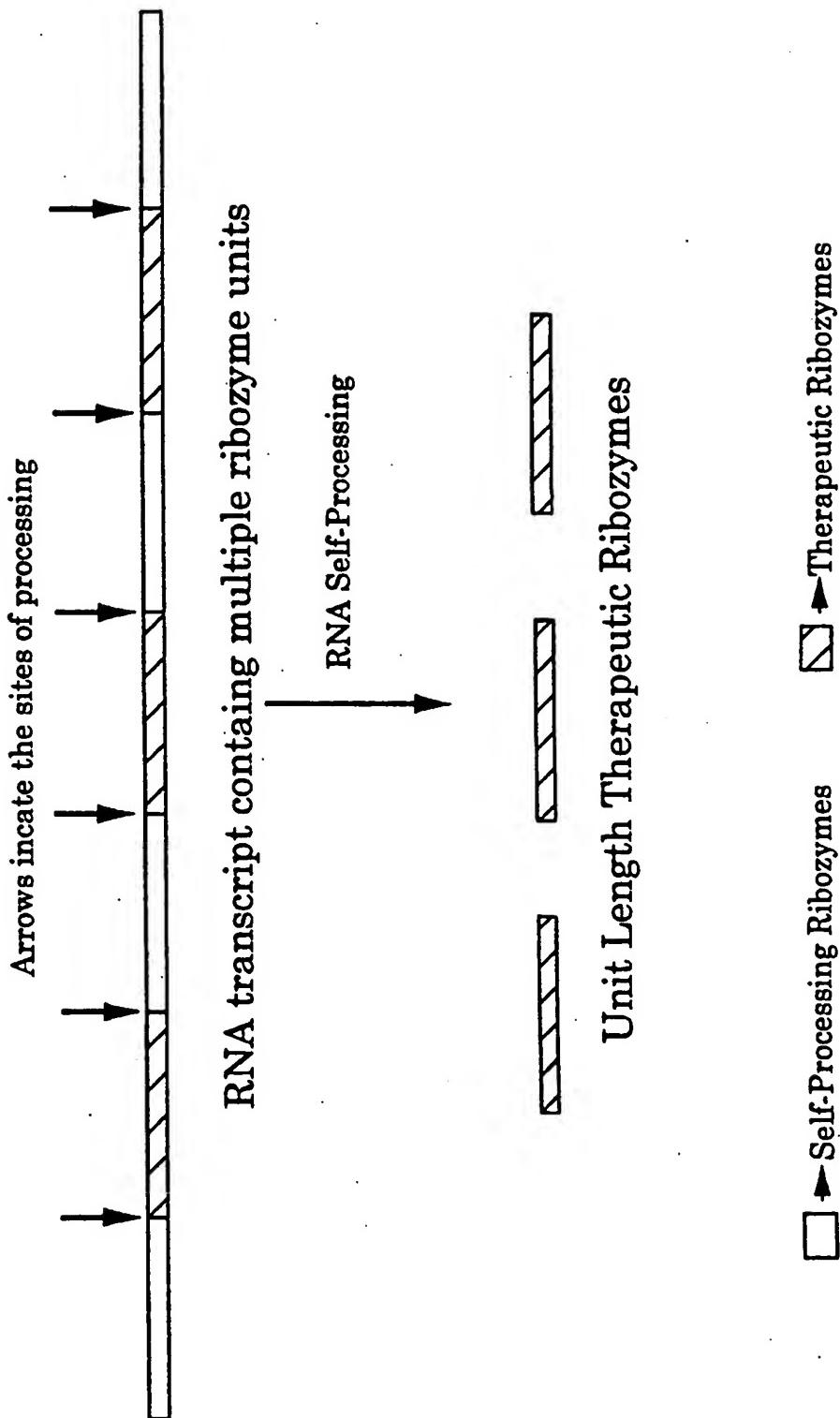


FIG. 106.

SUBSTITUTE SHEET (RULE 26)

102/103

FIG. 107.



103/103

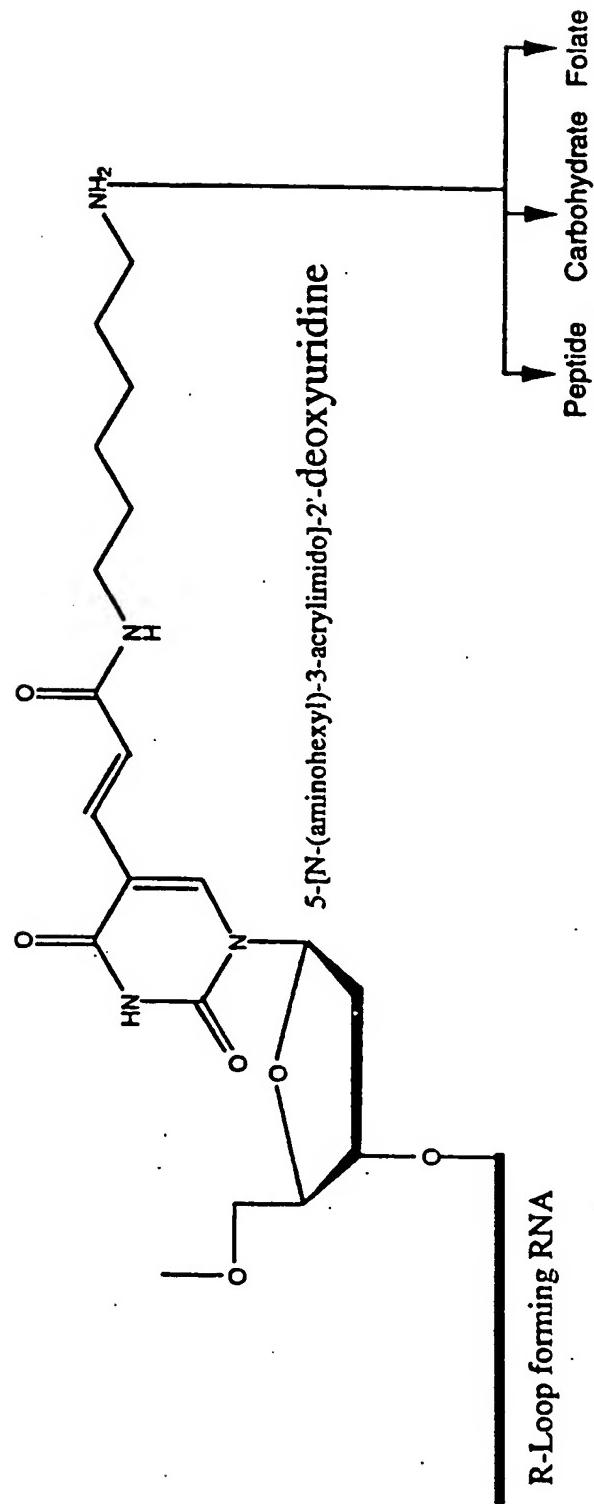


FIG. 108.